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THE VASCULAR SYSTEM OF THE RHIZOME AND LEAF-TRACE OF *PTERIS AQUILINA*, L., AND *PTERIS INCISA*, THUNB., VAR. *INTEGRIFOLIA*, BEDDOME.

BY
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AND
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[TEXT FIGS. 1-59.]

AS might be expected in the case of so widespread and abundant a plant, the structure of the vascular system in the common bracken fern has been frequently described and alluded to by plant-anatomists. Nevertheless, although it has long been known that both the inner and the outer rings of rhizome strands contribute to the formation of the leaf-trace, yet the exact course of the leaf-trace strands has apparently never been correctly described in adequate detail, nor has any attempt been made to bring the whole structure into its proper place in the modern theory of Filicinean vascular morphology. It is with the object of attempting to fill this gap that the present paper has been written.

HISTORICAL.

The earliest description we can find is that given by Hofmeister ('57) who made a fairly thorough investigation of the morphology and development of this plant. He describes how the single central vascular strand of the stem of the young plant at the insertion of the first leaf has a deep infolding on one side (Taf. II., Fig. 6), while after a number of leaves have been developed and the young stem has forked this bay becomes larger (Taf. II., Fig. 7) and soon the original strand is separated into two parallel band-shaped bundles (Fig. 8), which may branch, the branches again running into the main bundle. So far, Hofmeister is confirmed by Leclerc du Sablon ('90) and Jeffrey ('00). When the length of the primary forks of the rhizome have reached about three inches, continues the description, weaker bundles branch off from the two big ones and run further out in the cortex. The uppermost of these, running above the axile bundles, is developed more strongly, almost equalling the axile ones in breadth (p. 620). The stem of a fully grown plant shews in the main the same distribution of bundles. The number of peripheral ones may rise to twelve, of which the dorsal is always the largest. The roots arise exclusively from the outer bundles.

This description of the origin of the two circles of bundles in the young plant was first shewn by Jeffrey ('00) to be incorrect. In reality the two flat strands into which the original vascular cylinder of the stem divides are not equivalent to the two similarly shaped inner strands of the adult rhizome, but give rise by branching in a tangential direction to the *outer ring* of strands, while the two inner strands are successively nipped off dorsally from the inner face of the ventrally situated outer strand ('00, p. 10 and Pl. 7.)

With regard to the insertion of the leaf-trace in the adult plant, Hofmeister's description is the fullest that we are aware of, but is partly incorrect, while his figures are inadequate for a detailed appreciation of the relations of the different strands.

Stenzel ('61) corrects Hofmeister's account in one respect. He points out that the two circles of vascular strands are often in connexion by means of cross branches passing between the two plates of sclerenchyma from the ventral inner strand to the lateral members of the outer circle (Taf. 5, Figs. 6, 17), as well as by means of occasional cross branches passing through the dorsal sclerenchymatous plate from the dorsal inner to the dorsal outer vascular strand (Taf. 5, Fig. 8), and sometimes by similar strands passing through the ventral plate (Fig. 9). He also notes that in some weak stems both the two sclerenchymatous plates and the two vascular strands of the inner ring form a closed cylinder (Fig. 12). Stenzel's description of the origin of the leaf-trace is quite general, and his figures, like Hofmeister's, fail to convey a clear and detailed picture of the facts. We cannot bear out his statement that before the leaf has separated from the stem, the foliar vascular strands are all thread-like (cylindrical).

Mettenius, at the close of his well-known paper on *Angiopteris* ('63) devotes a few words to this species, and remarks that when the lateral bud formed on the base of the petiole develops late, the vascular bundles of both petiole and lateral shoot are almost completely separate, while if the development of the branch precedes that of the leaf and takes place higher on the petiole, then the stretch at the base, common to leaf and branch, has a structure like that of the main axis. We shall refer to these points later.

De Bary in his "Comparative Anatomy" ('77) repeats the leading points of Hofmeister's and Stenzel's descriptions, placing *Pteris aquilina* among the ferns with accessory cortical bundles, but adds nothing to our knowledge of the subject. Conwentz ('75) also follows Hofmeister, and does not advance the subject.

In 1890, Leclerc du Sablon ('90) in his well-known paper "Sur la formation de la tige dans les Fougères" described the transition from the primary root to the stem, up to the point at which the vascular system of the latter has the form of two curved plates. Leclerc du Sablon did not follow the development of the young plant to a more adult stage, but his figure of the two plates recalls so strikingly the two inner strands of the adult rhizome as to lend great colour to Hofmeister's erroneous description of the inner strands of the rhizome being directly continuous with these two primary ones, while the outer circle represented accessory cortical strands arising from the inner by branching. This conclusion was however, as already mentioned, entirely upset by Jeffrey's account ('00) of the behaviour of the vascular system of the young plant above the level at which Leclerc du Sablon's figure was taken. This shewed quite clearly that the ventral primary strand traced upwards, gives off two fresh strands in succession from its internal face, and *that it is these latter which are continuous with the internal strands of the rhizome*, while the two primary strands gradually break up at a higher level to form the outer circle.

Now while this discovery brings *Pteris aquilina* into line with the majority of ferns having a polycyclic vascular structure, in so far as it turns out to be the outer ring of strands which is the primary one, corresponding with the single ring of monocyclic ferns; yet the exceptional fact that about half (sometimes more, sometimes less) of the leaf-trace arises from the inner (secondary or additional) strands, remains to be explained. It is clear that in the hypothetical ancestor with a single vascular cylinder, the whole of the leaf-trace must have arisen from this, and it is the business of the morphologist to try to discover the steps by which the supplementary supply to the leaf originated.

The relation of the leaf-trace to the vascular system of the stem in *Pteris (Litobrochia) incisa*, Thunb., var. *integrifolia* we believe may give the clue to the solution of this difficulty.

PTERIS INCISA.

Our specimens of this plant were gathered in the woods near the top of Gunong Hijau in Perak (Malay Peninsula). They agree very well with three specimens in the Kew Herbarium, one collected by Scortechini and two by L. Wray, junr., all three also from Perak. The variety differs from the type of this very variable species in the fact of its pinnules being entire instead of wavy-edged or even pinnatifid.[1] The texture of the leaves, in our plants at least, is also more coriaceous than in the type and the plant appears to have longer and more straggling fronds. The anatomical structure of the node of our variety is also, as we shall see, considerably more complicated than in the typical *P. incisa*.

Gwynne-Vaughan ('03) in his recent important paper on the Anatomy of Solenostelic Ferns has described this species as possessing a typical solenostele from which is given off a curved leaf-trace directly facing the median dorsiventral plane of the rhizome (pp. 691-2). Since the striking complications connected with the insertion of the leaf-trace in our plant are not mentioned in Vaughan's account, he has been good enough to lend us some of his preparations to compare with our own (Fig. 4). From this comparison it is evident that there are wide differences between the vascular structure of the type and that of the variety.[2] Thus the internodal solenostele has a considerably greater diameter and a more wavy outline in the variety, while the leaf-trace is much larger in proportion even to the larger stem-stele and has a far more complicated outline. There are also certain histological differences, and from their anatomy one would scarcely suspect the two plants of belonging to the same species. Considering however the great differences that have come under our own observation in the anatomy of one and the same species, *e.g. Matonia pectinata*, *Lindsaya orbiculata* type, and var. *tenera*, we are not prepared to say that either of the plants under consideration has been wrongly

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[1] *See* R. H. Beddome, Ferns collected in Perak and Penang by Mr. J. Day. Journ. of Bot. 26, 1888, p. 2; and Beddome, Scortechini's Malayan Ferns. *Idem*, 1896, p. 225.

[2] We are not prepared to say that the differences of vascular structure between our plant and Gwynne-Vaughan's are constant as between the type and this variety. The species is an extremely variable one, sometimes possessing leaves of enormous length, and the larger leaved individuals other than those belonging to our variety may well show similar deviations from Vaughan's simple structure.

assigned. In the specimen of which we have spirit-material, two nodes were preserved, and at each of these the stem dichotomised, the stout petiole coming off between the two branches and forming a direct continuation of the part of the stem behind the fork.

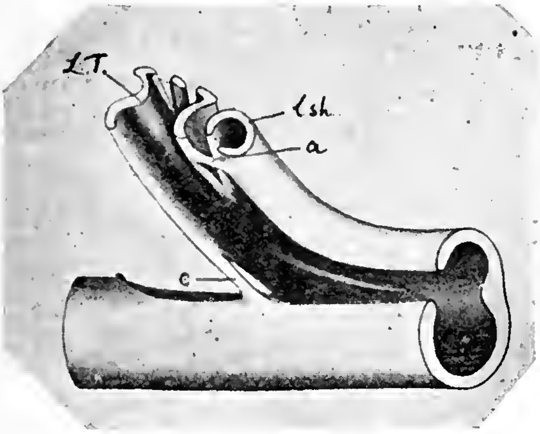


Fig. 1.

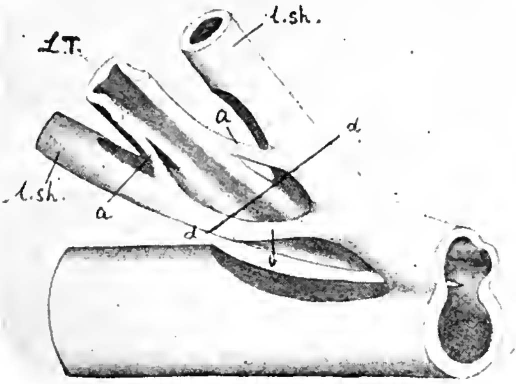


Fig. 2.

Fig. 1. *Hypolepis tenuifolia.* Diagram of vascular system of node seen from dorsal side. *L. T.*, leaf-trace. *l.sh.*, stele of lateral shoot. Fig. 2. Ditto of *Hypolepis repens.* *a*, *b*, *c*, *dd*, see text. (From Gwynne-Vaughan, l.c.)

In one case a shoot-bud also arose from one side of the petiole near its base, while in the other two such buds arose. Figs. 5-12 represent selected sections from a series through one of the two nodes (which were exactly alike), while Fig. 3 is a reconstruction of the stem-steles and the petiolar strand. From these it will be seen that while the stele of the internode is a solenostele, rather flattened in the horizontal plane, and wavy on the ventral side, from which roots are given off (Fig. 5), complications arise as the node is

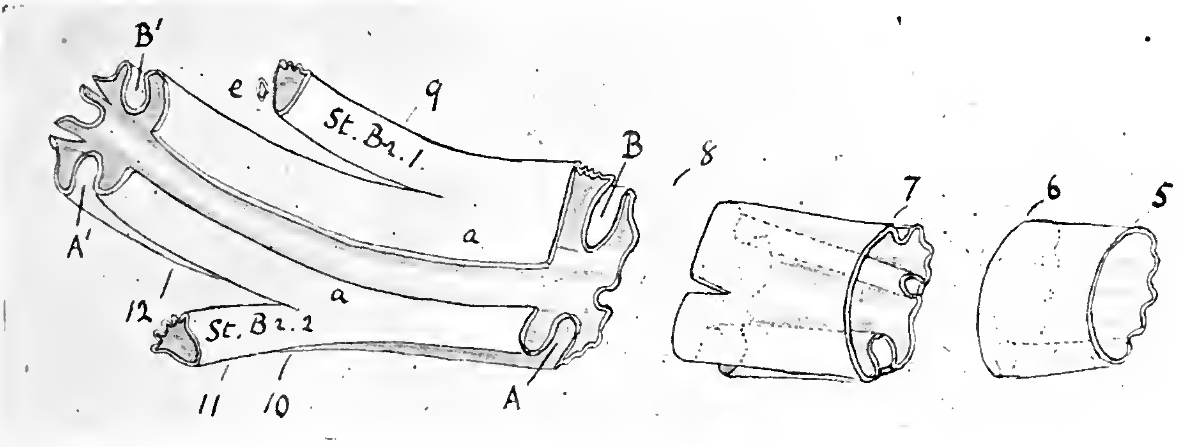
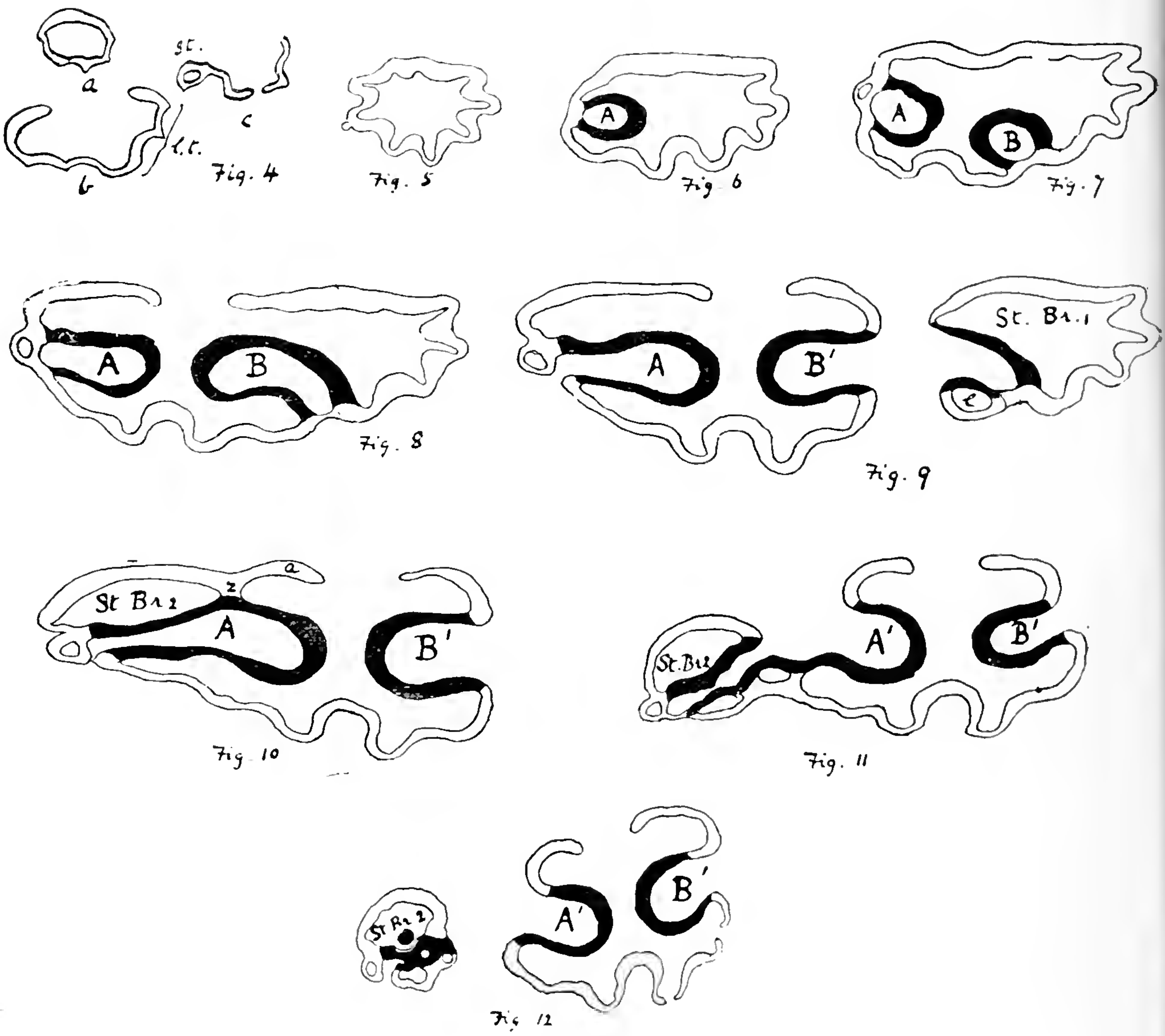


Fig. 3.

Fig. 3 *Pteris incisa* var. *integrifolia.* Diagram of vascular system of node seen from dorsal side. A, B, the two lateral folds of the stele. A^1, B^1, ditto of leaf-trace. *St. Br.*$_1$, *St. Br.*$_2$, steles of branches of dichotomy. 5—12, planes of section of figures 5—12. *a*, *e*, see text.

approached. First an internal fold of the solenostele having the form of a closed cylinder, arises on the left side (Fig. 6, A), and shortly afterwards a similar one arises to the right of the mid-ventral line (Fig. 7, B) and shifts up till it occupies a lateral position opposite the first. At the same time both of these internal cylinders



Figs 4.—12.

Fig. 4 (×6). *Pteris incisa*, type. *a*, solenostele of internode. *b*, solenostele broken for departure of leaf-trace, *l.t.* *c*, base of petiolar strand (of another leaf) in transverse section giving off the stele *(st.)* of a lateral shoot. From Gwynne-Vaughan's preparations.

Figs. 5—12 (×6). *Pteris incisa* var. *integrifolia*. Selected sections from a series through the node. Vascular tissue of the two lateral internal folds (A, B) and the tissue continuous with them above, *black*. A^1, B^1, lateral folds of petiolar strand. *St. Br.*$_1$, stele of first branch of the dichotomy. *St. Br.*$_2$ stele of second branch of ditto. *a*, *z*, *e*, see text.

become open to the exterior so as to form two deep lateral bays of the stele, and the whole structure increases greatly in size. Meanwhile the vascular ring has broken in the mid-dorsal line (Fig. 8). The central ground tissue now has the form of an irregular H lying on its side, closed in all round, except in the mid-dorsal line, by the vascular ring. The dorsal limb of the H is rather longer than the ventral one.

The right-hand end of this dorsal limb, which has retained the waviness of outline of the original stele, is now nipped off as a closed ring and becomes the stele of one of the branches of the stem (Fig. 9, *St.* $Br._1$). It is contributed to by a small vascular branch nipped off as a closed ring from the right-hand end of the ventral limb of the H. This quickly becomes attached to the ventral side of the branch stele, but retains its individuality for some distance (Fig. 9), eventually fusing completely with the stele to form one of the bays of its wavy margin.

The left-hand end of the dorsal limb of the H is now nipped off in the same way (Fig. 10) and becomes the stele of the other branch (*St.* $Br._2$) ; like the stele of the former branch it is also contributed to by a strand arising from the left-hand end of the ventral limb of the H. This strand breaks up into two before joining the stele of the branch (Fig. 11.). The third strand, lying on the ventral side of this stele (Fig. 12), has taken its origin previously from the outer side of the internal fold (A) of the original solenostele (Fig. 7). The three strands now join the ventral side of the branch stele and form the three central projections of the wavy ventral side (Fig. 12).

The abbreviated H (Fig 12) is now the petiolar meristele. It is to be noticed that the ventral side of its ventral limb and the dorsal side of its dorsal limb (left white in the figures), *i.e.* the parts corresponding to the arch and the free edges of the primitive curved petiolar strand from which it must have been phylogenetically derived, are continuous with parts of the solenostele of the stem below, while the deep lateral bays A^1 and B^1 (black in the figures) are continuous with the folds (A and B) which arose in the solenostele as the node was approached.

The structure of the vascular system at this type of node, in which the leaf arises between the two branches of what is practically a dichotomy, is at first sight difficult to bring into harmony with a node at which the leaf-trace is directly inserted on the stele of an axis that is continued as such beyond the origin of of the petiole.

A careful comparison of the figures illustrating the structure of the node of *Pteris incisa*, var. *integrifolia* with Figs. 1 and 2, which are taken from Gwynne-Vaughan's paper on Solenostelic Ferns ('03), and represent the vascular structure of the nodes of *Hypolepis tenuifolia* and *H. repens* respectively, will facilitate the interpretation of the structure under consideration in terms of the morphologically simpler case.

The leaf-trace of *Hypolepis tenuifolia* (Fig. 1) will be seen to come off from the lateral surface of the solenostele of the rhizome as a curved and folded plate, with its concavity facing the median dorsiventral plane of the rhizome. The stele opens dorsally at first, but the leaf-gap becomes lateral as soon as the leaf-trace is free.

As the trace departs it becomes deeply infolded on the basiscopic side, and the infolding so formed soon becomes separated off as a gutter-shaped strand (*l. sh.*) which closes to a cylinder and is the stele of the lateral shoot arising on the posterior side of the petiole base. Just after the gutter-shaped stele becomes free, a small strand (*a*) runs from its free edge furthest from the leaf-trace to join the basiscopic edge of the latter. Now if we imagine the stele of the main rhizome to decrease considerably in size, so that its continuation and the lateral branch arising from the base of the petiole become of about equal importance, we should have a "false" dichotomy of the stem, with the petiole arising in the angle of the fork, exactly as in the two nodes of *Pteris incisa* var. *integrifolia*. The parallel is even closer, though not perhaps so legitimate, between the node of the latter and the petiole-base with its *two* lateral shoots in *Hypolepis repens* (Fig. 2) if we imagine the continuation of the main rhizome to be suppressed altogether. The general resemblance between this and Fig. 3 will be obvious at once if we consider the form of the leaf-trace in cross section just below the separation of the lateral shoot-steles (*i.e.* along the line *d*—*d* in Fig. 2 corresponding with Fig. 8 in *P. incisa* var. *integrifolia*). Here the trace is in both cases complicated by the two deep dorso-lateral gutter-shaped infoldings most of which form the two branch-steles. In *P. incisa* var. *integrifolia*, however, the branch-stele is nipped off as a cylinder from the outer part of the infolding, *i.e.* from the end of the dorsal limb of the H, by the union of the dorsal and ventral walls of this limb at *z* (Fig. 10) so that no gap occurs either in the branch-stele or in the adjacent part of the trace, while in *Hypolepis* the stele separates as a gutter-shaped strand whose outer edge is united with the edge of the trace by means of the strand *a*

(Figs. 1 and 2), corresponding with the union of the free margin *a* with the deep lateral bay of the leaf-trace below at *z* in Fig. 10. The contributions of the *ventral* limbs of the leaf-trace to the branch-steles in *Pteris incisa* var. *integrifolia* appears to have no parallel in *Hypolepis*. They are no doubt correlated with the much greater development of these ventral limbs in the *Pteris*.

The strength of the foregoing comparison is increased by the fact that the leaf-trace of *Pteris incisa* (type, Fig. 4, *c*) is of exactly the same form as that of the two species of *Hypolepis*, but unfortunately no node of the former shewing the complication involved by branching of the stem is available for comparison. The petiolar strand of *Pteris incisa* (type) and of *Hypolepis repens* and *tenuifolia* shews a slight bay or concavity on each side, but this is not decurrent into the stele of the rhizome. In *Pteris incisa* var. *integrifolia* on the other hand the petiolar strand is much more complex, and in correlation with this the solenostele of the rhizome does not become simple for some distance behind the node. The complication of the petiolar strand is mainly due to the much greater development of the two lateral bays (A^1, B^1 in Figs. 9—12 and Fig. 3) and these are continued for some distance back in the rhizome, first as bays, then as hollow cylinders attached to the inside of the solenostele, and finally as open bays again (A and B in Figs. 6—8 and Fig. 3), which eventually die out altogether.

This increased complexity of the leaf-trace is no doubt a response to an increased demand for vascular tissue by an increased leaf-surface, and has resulted in an increased complexity of the vascular structure of the stem behind the node.

PTERIS AQUILINA.

Let us now turn to *Pteris aquilina*. It is scarcely necessary to resume the vascular structure of the rhizome, the leading points of which have already been mentioned as described by Hofmeister and Stenzel, and are well-known to every elementary student. The connexion of the petiolar strands of the base of the petiole with those of the rhizome appears, however, never to have been accurately described.

A transverse section of the base of the petiole, apart from the complications introduced by the arising of a lateral branch, shews an arrangement of the vascular strands easily derivable from the strongly curved plate which Gwynne-Vaughan has shewn to be the primitive type of leaf-trace in solenostelic ferns. In the dictyostelic types, as he has remarked, the general outline of this is kept, though the plate is not continuous, but is resolved into a number of distinct strands.

The degree of complication of the curve varies very greatly in the species under consideration. Let us first take the elaborate petiolar system represented in Fig. 34, since this gives us the closest resemblance to *Pteris incisa* var. *integrifolia*.

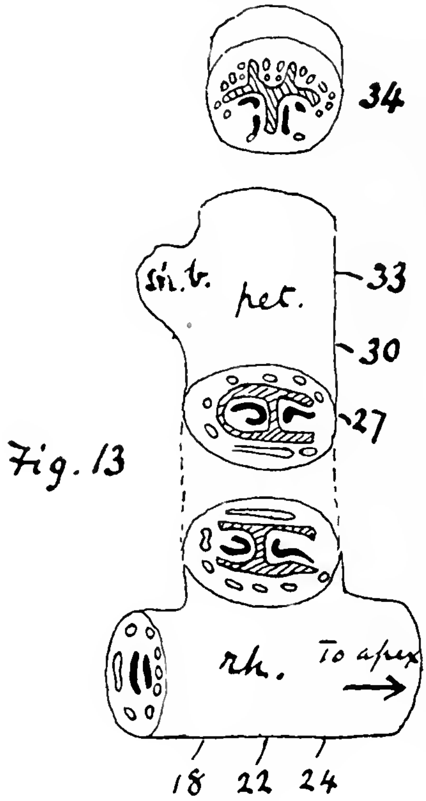
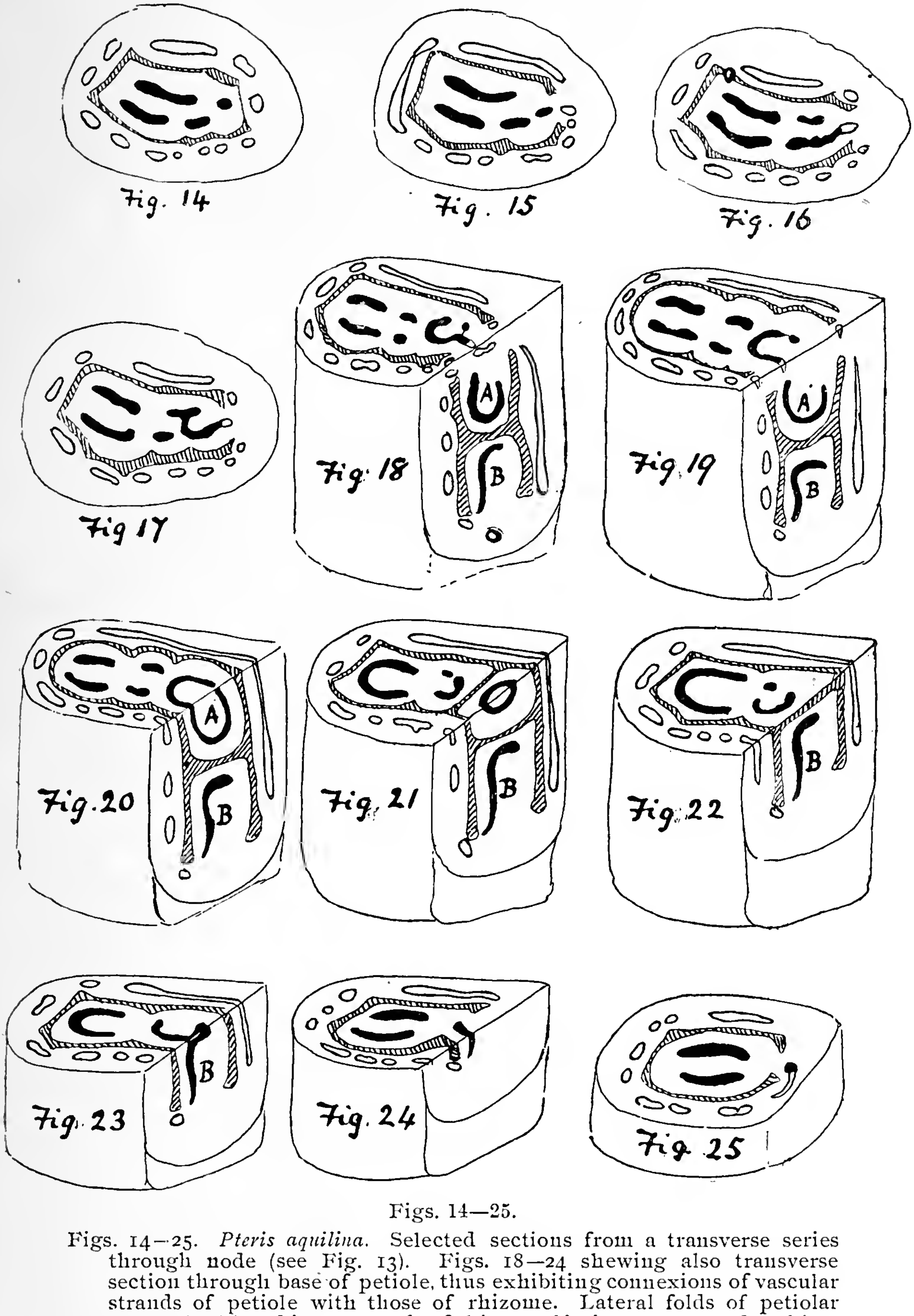


Fig. 13.

Fig. 13. *Pteris aquilina*. Diagram of node seen from ventral side. *rh.*, rhizome, *pet.*, petiole, *sh. b.*, shoot-bud. The numbers refer to the planes of section of the figures so numbered.

Fig. 34 shews the transverse section of the petiole just above the departure of the lateral branch (see Fig. 13). The petiolar strands are arranged on a figure of the general outline of an Ω with the top (abaxial side, bottom in Fig. 34, p. 12) pushed in. The resemblance to Fig. 12 is very close, the only difference in the outline of the figure being the absence of the incurvings of the free margins in Fig. 34. Furthermore the origin of the strands of the lateral shoot from those of the petiole, as shewn in Figs. 26—33 is strikingly similar to that of *Pteris incisa* var. *integrifolia*, if allowance be made for the fact that the internodal vascular system of *Pteris aquilina* consists of two circles of strands, while that of *Pteris incisa* is a simple solenostele. Compare Fig. 26 with Fig. 8, and Fig. 31 with Fig. 9.



Figs. 14—25.

Figs. 14—25. *Pteris aquilina.* Selected sections from a transverse series through node (see Fig. 13). Figs. 18—24 shewing also transverse section through base of petiole, thus exhibiting connexions of vascular strands of petiole with those of rhizome. Lateral folds of petiolar system (A, B) and inner strands of rhizome, *black*; outer strands, white; sclerenchyma, diagonally lined.

The strands represented in black in these figures are those which are continuous with the *inner* strands of the main stem, as may be seen in Figs. 14—25 (which represent a series of transverse sections of the rhizome passing through the node, the transverse section of the base of the petiole, in a plane at right angles to the parallel planes of the series, being also shewn in Figs. 18—24), while the strands left white are those derived from the outer circle of the rhizome. From an inspection of these series it is quite clear that the strands forming the lateral bays (A, B, A^1, B^1,) of the petiolar system are continuous with the inner strands of the rhizome, while the whole of the abaxial curve together with the free margins of the petiolar system are continuous with the outer strands of the rhizome. Thus while in *Pteris incisa* var. *integrifolia* the continuations backwards of the lateral bays of the leaf-trace gradually die out in the rhizome, in *Pteris aquilina* they form an inner system of rhizomic vascular strands continuous throughout the internodes, for

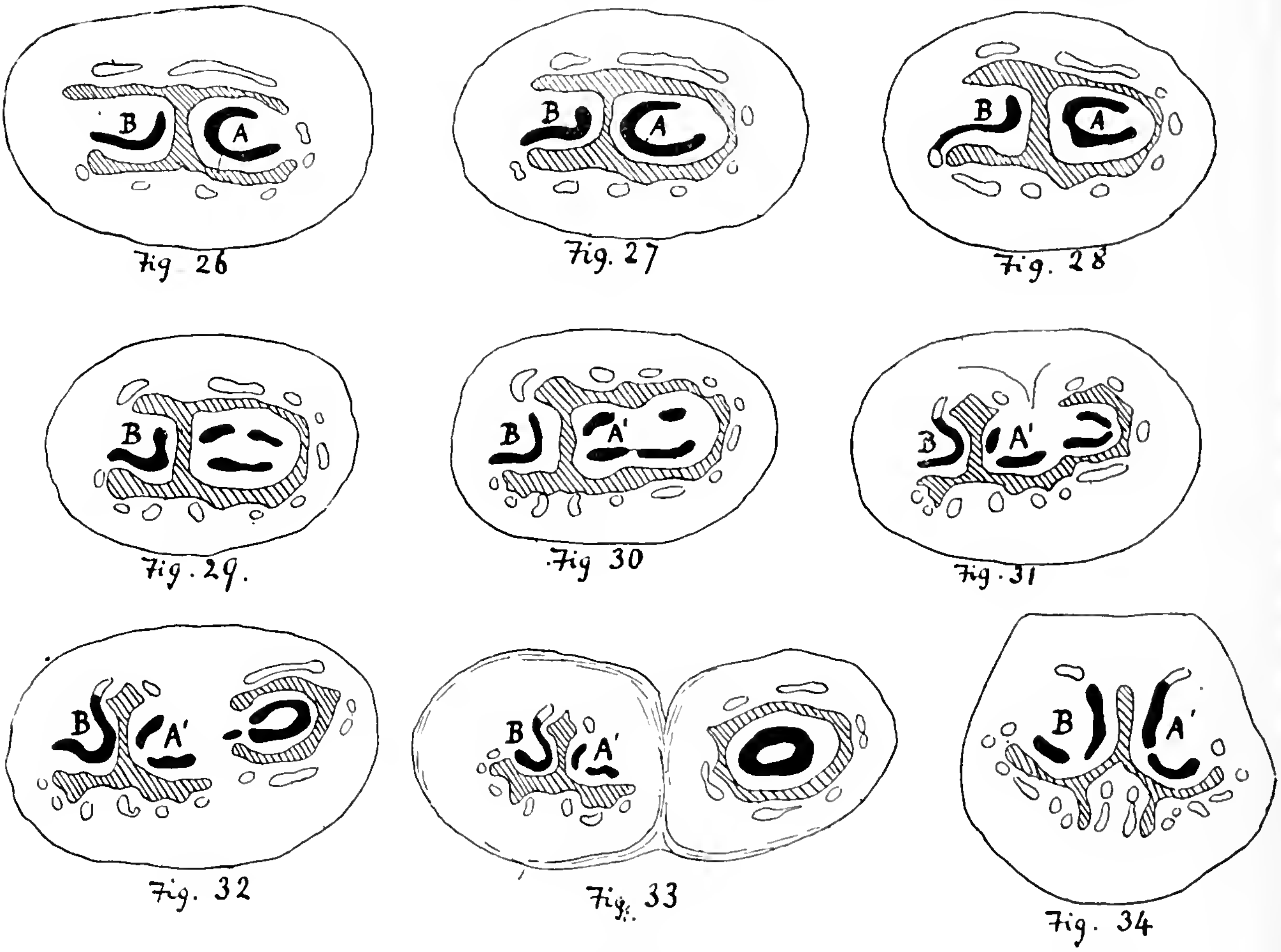


Fig 26 Fig. 27 Fig. 28
Fig. 29. Fig 30 Fig. 31
Fig. 32 Fig. 33 Fig. 34

Figs. 26—34.

Figs. 26—34. *Pteris aquilina.* Selected sections from a transverse series through the base of the petiole including the origin of a lateral (posterior) bud (see Fig. 13). Lateral folds of petiolar system (A. B,—above bud A^1, B) and inner vascular ring of bud, *black;* dorsal (adaxial) and ventral (abaxial) strands, *white:* sclerenchyma diagonally lined.

the most part independent of the outer primitive system, but, as Stenzel shewed, having occasional connexions with it. It is noteworthy that these connexions, according to Stenzel, are mainly, though not exclusively,[1] *lateral*, *i.e.* in much the same position as the connexion between the internal cylinders and the solenostele in *Pteris incisa* var. *integrifolia* (compare Figs. 16 and 50, with Figs. 6 and 7). Figs. 36—57 represent selected sections from a series through two successive nodes of the rhizome diagrammatically represented in Fig. 35. The petiolar system is in this case considerably simpler than that of the node represented in Figs. 14—34, and hence its resemblance to *Pteris incisa* var. *integrifolia* is much less marked, but it will be seen that the relations of the petiolar and rhizomic strands is identical with that already described. These relations in fact appear to be perfectly constant.

It is difficult to resist the conclusion that the vascular system of *Pteris aquilina* has passed through a stage like that now represented by *Pteris incisa* var. *integrifolia* on the way from its monocyclic ancestor. It does not of course follow that this intermediate stage was solenostelic; dictyostely may have arisen at any point in the series.

A few words must be devoted to the relation of the insertion of the leaf-trace to the branching of the rhizome. Two distinct types of branching exist in this species, first, ordinary dichotomy, in which the vascular system of the rhizome typically divides into two approximately equal parts,[2] and which has no necessary relation to the origin of a petiole; and secondly, the lateral origin of a shoot-bud from the posterior side of the base of a petiole (Figs. 13 and 26—33), in which the vascular system of the bud is derived entirely from that of the petiole. It is, however, very common for the weaker branch of the dichotomy to bear a leaf on the inner side more or less close to the fork of the rhizome. If the insertion of this leaf is sufficiently removed from the fork, the petiolar strands arise from the rhizome branch-system in the ordinary way. This seems to be Mettenius' second case, referred to above, but why he should consider the stretch from the base of the petiole to the fork as belonging to the *petiole* is not obvious. If the insertion of the leaf is closer to the angle, the strand forming the lateral fold of the

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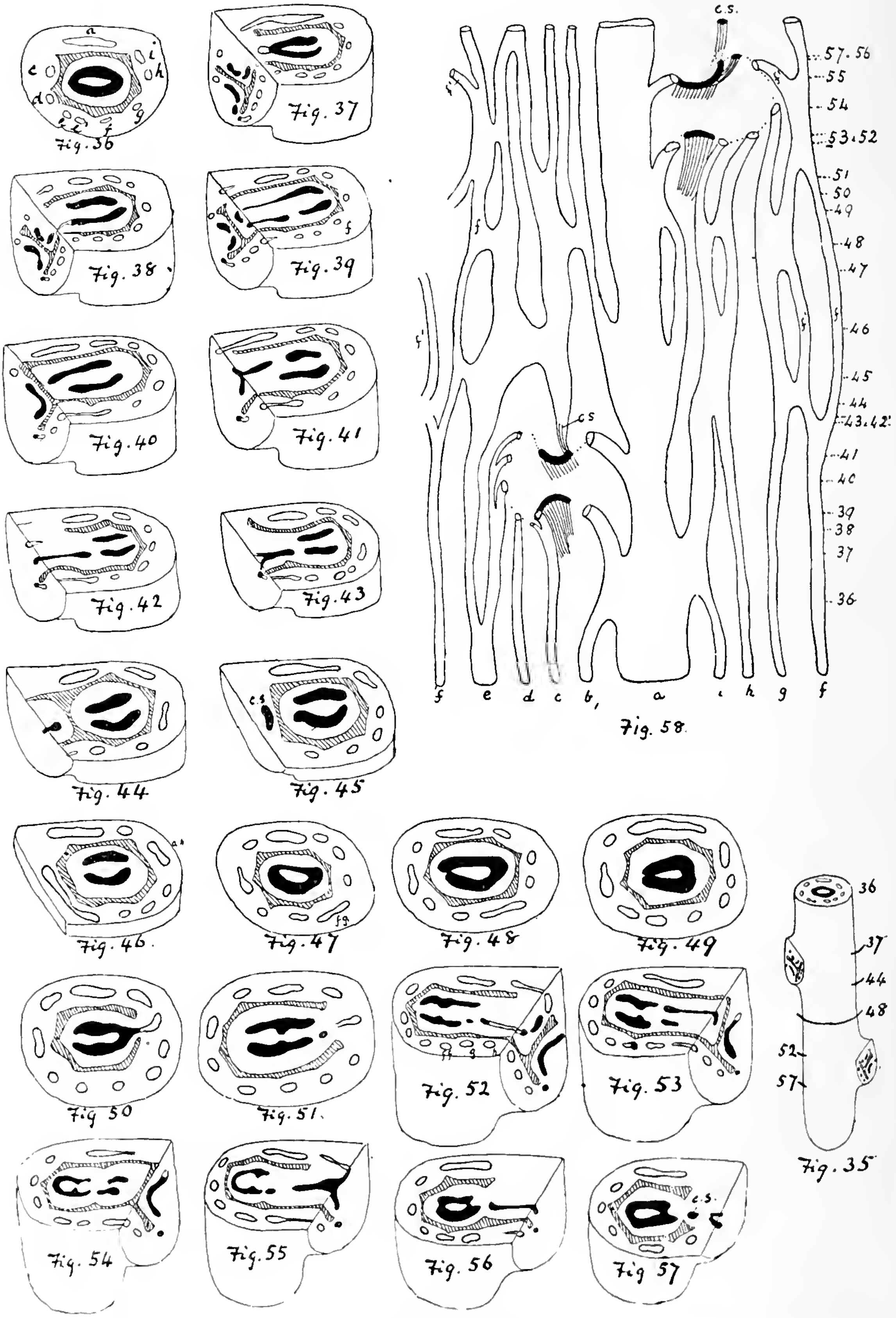
[1] We have not seen the dorsiventral connexions of the inner and outer strands figured by Stenzel ('61. Taf. V., Figs. 8 and 9), but it appears that they occur in larger rhizomes than those we have worked with.

[2] The division is however very often far from being equal, see below.



Figs. 35—58.

Fig. 35. *Pteris aquilina.* Diagram of piece of rhizome seen from ventral side with two nodes. The numbers refer to the planes of section of the figures so numbered.

Figs. 36—57. *Pteris aquilina.* Selected sections from a transverse series through the piece of rhizome represented in Fig. 35, shewing the departure of two successive leaf-traces. Vascular strands and sclerenchyma represented as in previous figures. *c.s.*, compensation-strands, see below.

Fig. 58. *Pteris aquilina.* Diagram of the course of outer vascular strands of piece of rhizome in Fig. 35 represented as spread out in a plane, the dorsal strand in the middle. Sections of the leaf-trace strands are shewn where they pass out of the plane of section, those derived from the inner strands of the rhizome black. An inner strand *(c.s.)* passes into the outer circle as a "compensation-strand" and helps to close each leaf-gap.

petiolar system on the basiscopic side may arise directly from the inner strands of the rhizome behind the fork and run independently of the branch system, though the rest of the leaf-trace arises directly from the latter. This is a frequent case. Finally, if the leaf is inserted actually in the angle of the fork, its trace arises from the rhizome strands behind the fork quite separately from the branch. The last two types are apparently covered by Mettenius' first case.

It is noteworthy that the vascular system of the petiole, when it arises in the actual angle of the dichotomy, does not in this species ever become so important in relation to that of the rhizome as to form a direct continuation of the latter, the branches of the dichotomy being nipped off from its sides, as in the case of *Pteris incisa* var. *integrifolia*. On the other hand, though we have not found cases shewing intermediate vascular structure, it is easy to see how the weaker branch of the dichotomy which in this type is developed late and is much smaller than the stronger one, could, by a still further delay in its development, be carried up onto the petiole and so lead to the type in which a bud arises from the posterior side of the petiole base. In this way it is possible to suppose that this type of branching, not uncommon in ferns, may have been derived from primitive dichotomy in which a leaf arose on the inside of one of the branches. This appears to have been Stenzel's view ('61, p. 40 and footnote). In any case it is significant of some genetic connexion that where in this species there is branching in the neighbourhood of leaf-insertion, the relation of position between the stronger branch, the weaker branch and the leaf is constant. The whole subject of the morphology of branching in ferns and its relation to vascular structure—a subject which occupied a considerable amount of attention about the middle of last century—deserves re-investigation.

Fig. 58 is a representation of two nodes and the intervening internode, the cylinder formed by the outer rhizomic system being spread out on a plane; the inner strands (black in section) are only put in where, at the nodes, they pass through the plane of the cylinder. If this figure be compared with the similar figures of Mettenius it will be readily seen that the outer rhizomic system is a much dissected dorsiventral dictyostele in which there remains one constant broad dorsal strand, representing a dorsal strip of the original solenostele, as for instance in *Polypodium latipes* (Mettenius '63, Taf. IX., Fig. 6). See Gwynne-Vaughan ('03, pp. 694—698).

We conclude, therefore, that the vascular system of *Pteris aquilina* is a dorsiventral dictyostele of the Polypodium-type with an internal system of accessory strands developed in connexion with lateral elaboration of the leaf-trace.

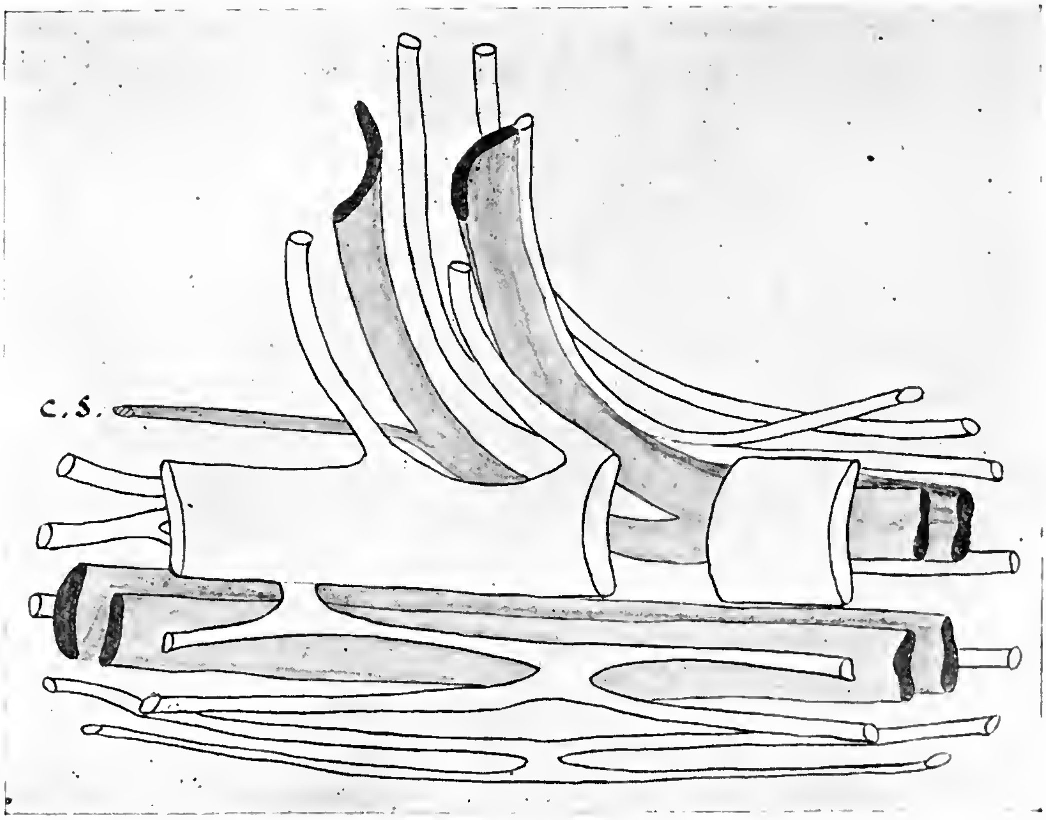


Fig. 59,

Fig. 59. *Pteris aquilina.* Diagram of the course of the vascular strands at a node, somewhat simplified, seen from the dorsal side. Inner strands of rhizome and lateral folds of leaf-trace shaded. A part of the dorsal outer strand is supposed to be removed, thus shewing the branching of the inner ones. *c.s.*, compensation-strand.

Fig. 59 is a diagram of the vascular system of the node of *Pteris aquilina*, drawn for comparison with Figs. 1—3. It is represented as somewhat simpler than any node we have actually seen, so as to avoid confusion. The inner strands of the rhizome and the petiolar strands in connexion with them are darkly shaded.

The probability of the modification of vascular structure at the node of a plant, in relation to an alteration of the leaf-trace, and the effect of leaf-traces in modifying stem-structure generally, has been pointed out in one form or another by nearly all recent writers on vascular morphology. In ferns the principle of the decurrency of such a new structure from the node into the internode below, and its eventual establishment throughout the internode to join a similar structure at the next node below was tentatively suggested by Boodle ('01, pp. 737-8) and by ourselves in reference to *Lindsaya* and *Davallia pinnata*.[1] These suggestions have been confirmed and greatly extended by Gwynne-Vaughan ('03). There can now be little doubt that we have here a principle of very wide application, at least within the Filicinean series. The particular instance of it described in the present paper is of special interest, since it brings before us a mode of origin of an internal system of rhizomic vascular strands differing from that described by Gwynne-Vaughan in a number of types. Whereas the internal ridges and strands in the Dicksonias, in *Cyathea*, and in *Pteris elata* var. *Karsteniana* arise at the node as a local thickening of the leaf-gap, those of the plants we have described arise as lateral elaborations of the leaf-trace itself.

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[1] Tansley and Lulham. On a New Type of Fern-Stele, Ann. of Bot., XV., 1902.

University College, London. *December, 1903.*

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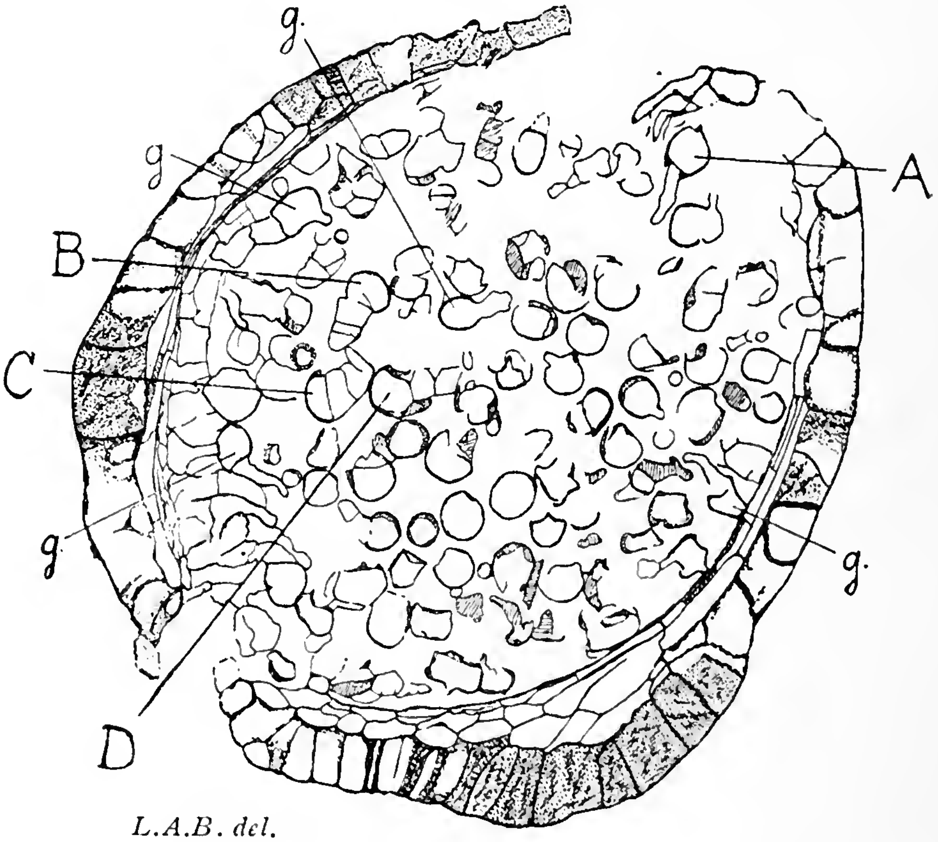
GERMINATING SPORES IN A FOSSIL FERN-SPORANGIUM.

BY D. H SCOTT, F.R.S.

[TEXT FIGS. 60 AND 61.]

SPORANGIA of Ferns are of fairly common occurrence as petrified specimens in the calcareous nodules from the Lower Coal-Measures of Lancashire and Yorkshire. A few examples have been described and figured by various authors,[1] but no comparative investigation of these fossils has yet been undertaken. The need for such work is becoming urgent, even apart from other



L.A.B. del.

Fig. 60.

Fig. 60. General view of the sporangium. A, B, C, D, the germinating spores shown in detail in Fig. 61. *g*, other examples of stages of germination. ×69.

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[1] Carruthers, Notes on some Fossil Plants, Geol. Mag. 1872, p. 3, Fig. 5; Williamson, Organisation of Fossil Plants of Coal-Measures, Part VIII., Phil. Trans. 1877, p. 219, Figs. 25-30; Kidston, Fructification and Internal Structure of Carboniferous Ferns, Trans. Geol. Soc. Glasgow, Vol. IX., 1889, p. 13, Figs. 11–13; Bower, Is the Eusporangiate or the Leptosporangiate the more primitive Type in the Ferns? Ann. of Bot., Vol. V., 1891, p. 124, Plate VII.

reasons, in order that we may be able to distinguish between the sporangia of actual Ferns and the pollen-sacs of allied seed-bearing plants. In the meantime the object of the present note is to record a case in which there happens to be convincing evidence that the fructification in question is that of a true Fern.

The sporangium about to be described occurs in a section (No, 1832 in my collection) received from Mr. J. Lomax in Feb. 1903, and cut from a nodule obtained from the Halifax Hard Bed. The sporangium, as it appears in the section, is somewhat pear-shaped (Fig. 60) measuring 1·2 mm. in length, and about 1 mm. in maximum width. There are unfortunately no traces of the organ on which the sporangium was borne; its point of attachment may not improbably have been near the smaller end, but there is no reason to assume that the section is a truly longitudinal one. The sporangial wall, which is broken at both ends, consists of an outer, dark layer of cells, and an inner, more delicate zone, from one to four cells thick, as preserved, and in places much collapsed. In the multiseriate structure of its wall the sporangium resembles those of the Eusporangiate Ferns. The outer layer shows a certain differentiation; towards the smaller end and along the sides of the sporangium the cells are long and somewhat flat, their radial depth being only 50-60μ, while their length in the tangential direction reaches 100μ; towards the opposite end the form of the cells changes—they become high and narrow, the radial dimension reaching 110μ or more, while in the tangential direction they measure only from 30-50μ. In a section of a similar sporangium from Dulesgate, probably of the same species (S. Coll. 1831), the region occupied by the high, narrow cells is more extensive, embracing more than half the circumference of the sporangium; the two regions pass over gradually into each other. In this specimen the height of the narrow cells reaches 160μ. There is no sign that the apparent height of the cells is exaggerated by obliquity of section, and in any case there can be no doubt of the existence of a well-marked differentiation in the sporangial wall. It is most unlikely that the high-celled part of the wall represents a true annulus; in all probability this modification affected a considerable area of the wall; this is a common condition among Palaeozoic Fern-sporangia (*Scolecopteris*, *Sturiella*, *Botryopteris*, etc.), and finds its analogy among recent Ferns in the group of enlarged cells which discharges the functions of an annulus in the Osmundaceae. Professor Bower has already pointed out the close agreement between certain Carboniferous sporangia and those of this recent family and especially of the genus *Todea*.[1] The comparison holds good equally for the specimens now under consideration, which are similar to, though probably not identical with some of those figured by Professor Bower (cf. especially his Fig. 4, from slide No. 1875, in the Williamson collection).

The sporangium shown in Fig. 60 contains a considerable number of spores, but they by no means fill the cavity; many had evidently escaped, and some of these are found loose in the surrounding matrix. The spores are approximately spherical, and have a diameter of 65-70μ. The interest of the specimen lies in the fact that a number of the spores had begun to germinate within the sporangium when fossilization took place. It is indeed not improbable that all had done so, though it is only in certain favourable cases that the stages of germination are clearly shown. Four of the best examples of germinating spores are represented, highly magnified, in Fig. 61. Their position in the sporangium will be seen on reference to Fig. 60.

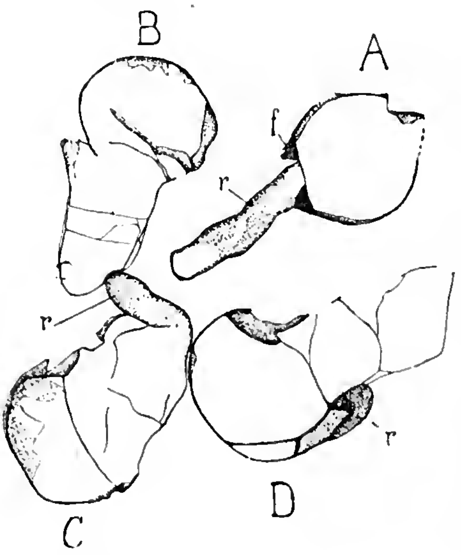

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Fig. 61.

Fig. 61. Four germinating spores in detail. (Cf. Fig. 60). A, *r*, rhizoid, *f*, flaps of exospore. B, shows prothallus only. C, shows prothallus and rhizoid, *r*. D. shows incomplete prothallus, and rhizoid *r*. × 195.

The spore A, 70μ in maximum diameter, has put out a process about 80μ long, and 22μ in maximum width; it is cut off by a septum from the cavity of the spore, and the flaps of the exospore (Fig. 61, A, *f*) where the outgrowth had burst through, are clearly seen. The relatively narrow outgrowth in this case was evidently of the nature of a root-hair or rhizoid; it is seen partly in section, and partly in superficial view.

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[1] Bower, loc. cit. p. 124, Plate VII.

The three spores, B, C and D, which are shown in juxtaposition as they actually occur (cf. Fig. 60), present other stages of germination. The spore B (72μ in greatest diameter) has produced a thick outgrowth, about 80μ long and 40μ thick in the middle part; it has split away somewhat on one side from the spore-membrane, and is cut off by a septum which is partly displaced towards the cavity of the spore. The outgrowth itself appears to consist of three cells, of which the middle one is very short ($13{\cdot}5\mu$). Both the transverse lines appear to represent genuine septa, though the frequency of cracks in the matrix demands caution. The lowest septum, which is somewhat broken, is undoubtedly a true cell-wall. The whole outgrowth is clearly a young stage of the prothallus.

Spore C, though slightly damaged, shows a very evident case of prothallial development. The outgrowth at its base is of the full width of the spore, from which it is separated by a septum, while a second transverse wall divides it into two parts of nearly equal length. At the narrow end of the young prothallus a rhizoid has grown out at a sharp angle; it abuts on the end-wall of the prothallus of B, and has somewhat indented it (Fig. 61, C, *r*). The arc of thick cell-wall seen in the angle between rhizoid and prothallus is probably a fragment of the exospore.

Spore D appears to have been more advanced in germination than the others. Here the outgrowth, measured from the septum dividing it from the spore cavity, is about 85 μ long, but it appears to be incomplete. It consists of two cells, or three if we include the spore. The septa between the cells have partially split, and the last cell, from its form, could not have been really terminal, but one or more cells would appear to have been lost. A rhizoid (*r*) is seen, closely applied to the spore and prothallus; it takes a curved course, so that its upper and lower walls are partly seen in surface-view; the attachment of the rhizoid to the prothallus does not lie in the plane of section.

These four examples may serve to illustrate sufficiently the stages of germination observed. A large number of other germinating spores are present in the sporangium, as will be seen on examining Fig. 60, where some of the clearer cases are lettered (*g*), showing rhizoids and young prothalli. At several places the small round transverse sections of rhizoids are seen, belonging no doubt to germinating spores not seen in connection with them. As mentioned above it is not unlikely that every spore left in the sporangium had started growing.

The agreement between the stages met with in the fossil and those of recent germinating Fern-spores is manifest. Fig. 61 A is a typical early stage,[1] where only the rhizoid has developed. In B we have a normal young prothallus (a little younger than that shown in Kny's Fig. 4, l.c.) There may very probably have been a rhizoid here also, which the section has missed. In C the type of germination appears to have been that described by Campbell in *Osmunda Claytoniana*, where the exospore is pushed up with the growing prothallus;[2] a fragment, however seems to have been left behind, next the rhizoid. The spore D may represent Campbell's other type, where the exospore remains at the base of the prothallus, though we cannot be certain of this, as the attachment of the rhizoid is not shown.

The germination of spores within the sporangium is not uncommon in recent Ferns, when, from any cause, effectual dehiscence has been hindered. A good case (from *Pteris serrulata*) is figured by Atkinson.[3] That this had happened in the case of our fossil is a fortunate accident, for otherwise it is very unlikely that such delicate structures should have been preserved uninjured, or that they could have been recognized. The spores lying loose in the surrounding débris do not show any clear case of germination, perhaps owing to damage when no longer protected by the sporangial wall.

The observation of these germinating spores is of interest as showing that some at least of the Carboniferous Ferns followed the same course of development as their recent allies. The agreement with corresponding stages in the development of Fern-prothalli at the present day, leaves little doubt that in this Carboniferous Fern also the spores produced the sexual generation in the way familiar to us. It may be hoped that this form of sporangium may eventually be referred to the Fern on which it was borne. At present we have no satisfactory clue to its attribution; in the same preparation with the specimen described, a very beautifully preserved portion of

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[1] cf. Kny, Bot. Wandtafeln. Abth. IX., 1895. Taf. XCVI. Fig. 2 *(Aspidium Filix-mas)*.

[2] Campbell, On the Prothallium and Embryo of *Osmunda Claytoniana*, etc., Ann. of Bot., Vol. VI., 1892. p. 52, Pl. III., Fig. 6.

[3] Biology of Ferns, 1894, Fig. 12. Mr. Boodle reminds me that he observed spores germinating in sporangia of *Todea hymenophylloides*. "which were ruptured but still arranged in sori on the lower side of the leaf." Anat. of Hymenophyllaceae. Ann. of Bot., Vol. XIV., p. 484, 1900. The possible affinity of our fossil with Osmundaceæ gives this case a special interest.

frond, of the *Sphenopteris* type, is shown in superficial section, but there is no evidence to connect it with our sporangium. However this may be, it is a distinct point gained, to know that this form of sporangium, at any rate, was that of a true Fern.

In conclusion I desire to express my thanks for help given by my wife, who was the first to notice the germinating spores, and by Mr. L. A. Boodle F.L.S., who has taken much kind trouble in preparing the illustrations.

ON THE FERTILISATION, ALTERNATION OF GENERATIONS AND GENERAL CYTOLOGY OF THE UREDINEÆ.

By Vernon H. Blackman, M.A.

(Preliminary Notice).

THE question of the sexuality of the Uredineæ has been a disputed one ever since the suggestion put forward by Meyen more than sixty years ago that the spermogonia and aecidia represented male and female organs respectively. Further observations on the usual close association of these structures and the apparent absence of power of germination of the spermatia seemed at first to confirm this view; still later investigations, however, failed to trace a process of fertilisation or even to discover any clear evidence of sexual organs in the aecidia. Such results, combined with the observation that the spermatia were under certain conditions capable of germination to a slight degree, led Brefeld to put forward the view, accepted by many botanists, that sexual organs were wanting in the Uredineæ and that the spermatia were nothing more than conidia. A great objection to this view, however, was the fact that the spermatia seemed quite incapable of causing infection, and germinated feebly only under conditions (highly artificial for true parasites like the Uredineæ) of nutritive solutions.

The cytological observations of Poirault and Raciborski and of Sapin-Trouffy about 1896 threw very little light on the spermatia, the conidial nature of which was assumed. These workers, however, showed that a very peculiar condition was to be observed in many stages of the Uredineæ, in which the nuclei were always to be found closely associated and dividing in pairs (the so-called conjugate nuclei) and arranged in binucleate cells. Sapin-Trouffy carried the matter further, and stated that the following, very interesting cycle of nuclear development, was to be observed in the Uredineæ having the requisite number of spore-forms. The mature teleutospore is uninucleate and gives origin to uninucleate sporidia from which a mycelium with uninucleate cells arises (*i.e.* the nuclei are single); on this mycelium spermogonia with uninucleate spermatia arise, but in the young aecidium the nuclei become paired with the result that the aecidiospores contain two nuclei. This paired condition of nuclei is then retained throughout the ensuing mycelia and uredospores up to the teleutospores, which in the young state are binucleate, but later become uninucleate by the fusion of the two nuclei. This process of fusion Sapin-Trouffy considered to be of the nature of a true fertilisation.

In these important observations, however, very little attention was paid to the spermatia or to the exact method of transition from the condition of single to that of paired nuclei, a very peculiar method of nuclear division was also described and the figures were of a somewhat diagrammatic nature. It thus seemed important that the life-cycle should be worked out in detail in a few forms, so during the last two years investigations have been carried out on *Phragmidium violaceum*, Wint. (probably identical with *P. Rubi* Wint.) and on *Gymnosporangium clavariaeforme*, Rees, with results which seem to throw very considerable light on the sexuality and life-history of the Uredineæ. A short account only is published here; the full paper, with figures, will appear later in the *Annals of Botany*.

The spermatia were carefully investigated in these two forms and they were found to possess very striking characters,—a thin cell-wall, no reserve material, a very large nucleus with no nucleolus, and cytoplasm greatly reduced in amount. These characters are certainly not those of conidia, but on the other hand are very clearly those of male cells. When the special cytological features of the spermatia are considered in connection with their close association with the aecidia,[1] their total lack of infecting power and

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[1] The appearance at about the same time as the very effective aecidiospores of organs so structurally ill-equipped for the purpose of infection as are the spermatia is certainly a strong argument against the conidial view.

their feeble power of germination (a total absence of power of germination is, of course, not a necessary character of male cells), the conclusion seems inevitable that the spermatia are male cells, which formerly played a part in connection with the aecidia, but have now become quite functionless.

The alternation of stages with single and paired nuclei was fully confirmed as was also the fact that the transition takes place in connection with the aecidium. The development of the very simple and probably primitive aecidium of *P. violaceum* was followed out in detail; it arises as a layer of rectangular uninucleate cells just beneath the epidermis of the leaf. Each of these cells becomes divided into a short *sterile cell* above, which soon degenerates, and a *fertile cell* below, which increases in size, becomes binucleate and gives origin to a row of binucleate aecidiospore-mother-cells, which divide and form binucleate aecidiospores and intercalary cells. Thus the condition of paired nuclei starts in the fertile cell and is continued to the teleutospore. The fertile cell, however, becomes binucleate, not by division of its original single nucleus, but by the migration through the wall of the nucleus of a neighbouring vegetative cell of the mycelium.

The fertile cell has the characters of a female cell; it cuts off a sterile cell, and it is stimulated to further active development by the entrance of a nucleus from without. It is accordingly impossible to consider this process other than as one of *fertilisation*, for the delay in fusion of the nuclei has its parallel among certain animal eggs (e.g. *Cyclops*). In the absence of spermatia this process might be looked upon as a simple form of ordinary fertilisation in which the male cell was undifferentiated and there was no complete fusion of the two cells. In the presence, however, of the spermatia with their special cytological characters, etc., the only view that seems capable of explaining the facts is that the fertile cell (=female cell) was formerly fertilised by the spermatia, but that now the process has become reduced, fertilisation by means of spermatia having been replaced by the more certain method of fertilisation by the nucleus of a neighbouring vegetative cell. This process is probably to be looked upon as intermediate between the normal process of fertilisation with differentiated male and female cells and that observed recently by Farmer, Moore and Digby in the case of apogamous ferns, where both acting male and female cells are ordinary vegetative cells.

If the view be accepted that the spermogonia represent male, and the aecidia collections of female reproductive organs in *P. violaceum*, and therefore in the whole group, it becomes clear that the Uredineæ present an alternation of generations which is as sharply marked as that of the higher plants. For not only can the two generations be distinguished as sexual and asexual, but they are also to be cytologically differentiated by the possession of single and paired nuclei, respectively. The gametophyte generation starts with the uninucleate teleutospore which seems to correspond with the spore-mother-cell, and like it is followed by a tetrad division by which the four sporidia are produced; from these the mycelium of the gametophyte develops and produces later the spermogonia and aecidia. The fertilised cell in the aecidium starts the sporophyte generation with paired nuclei, from which the aecidiospores almost immediately arise; the sporophyte mycelium bears later uredospores and finally teleutospores.

The fusion of nuclei in the teleutospore cannot then be looked upon as a process of fertilisation, but merely as the secondary process which brings about the disappearance of the special cytological conditions initiated by fertilisation, *i.e.* change from the sporophytic to the gametophytic condition; it must accordingly be looked upon as a *reduction-process*. Like the reduction-process in the higher plants it is followed by a tetrad division.

A careful study of nuclear division in the two forms in question shows, that in all the divisions except those in the promycelium, it is of such a simple, no doubt reduced, type that chromosome-formation is in complete abeyance; it becomes then impossible to assert that reduction in number of nuclei in the teleutospore is also associated with reduction in the number of the chromosomes. In the promycelium, however, the divisions are much more typical, for a well-marked spindle with centrosomes and polar radiations was observed, the spindle being developed between the centrosomes outside the nucleus and coming later into close connection with it. Only in the first division of the promycelium in *G. clavariaeforme* were chromosomes to be observed. The chromosomes were numerous; the two chromosomes observed regularly by Sapin-Trouffy and Maire, on which they base their evidence for reduction, represent groups of chromosomes or merely chromatin masses.

These conclusions as to the nature of the fusion in the teleutospore apply equally to the fusion of nuclei in the basidium, for it has lately been shown by Maire that the *Basidiomycetes* also have paired nuclei and that, as in the Uredineæ, it is the two individuals of a pair which fuse in the basidium. The exact point in the life-history at which the nuclei first become associated in pairs has not yet been ascertained for this group, but it is clearly there that one must look for evidence of a sexual process, and not to the reduction-process in the basidium. It is possible that the paired nuclei in this group may have their origin *apogamously*, by the association of the nuclei of two vegetative cells (cf. apogamy in ferns) or even by the association of two daughter-nuclei in one cell.

The forms among the Uredineæ in which the aecidium is absent and the nuclear association takes place in connection with the uredospores or teleutospores are probably also to be looked upon as cases of *apogamy*, but they require further investigation. The curious shortening of the life-history observed in *Endophyllum*, in which the aecidiospore with paired nuclei germinates as a teleutospore and the nuclei separate later to form uninucleate sporidia, may clearly be compared with cases of *apospory* among the higher plants.

REVIEW:

A MONOGRAPH OF THE TAXACEAE.

By R. Pilger

(Das Pflanzenreich, 18 Heft. Regni vegetabilis conpectus;
Edited by A. Engler).

THE group of Gymnosperms (we will not term them Coniferæ) known as Taxaceae has always seemed to us to be one upon whose affinities, morphology and structure the fullest light has never yet been thrown, and concerning which a great deal of ignorance still prevails. For this latter there is no longer any excuse. Before us lies a work containing every possible species of information about each of the members composing this very peculiar group. It is fashioned on the lines of "Die natürlichen Pflanzenfamilien," and is one of the sections of the great work "Das Pflanzenreich" edited by Engler.

The first part of the book, which, except for a Latin description of the characters of the group, is in German, opens with a brief bibliography of the subject and proceeds with a detailed and exhaustive account of the vegetative organs, anatomy, floral structure, fruit and seed, pollination, fertilisation, embryology, geographical distribution, economic uses, and relationships of the group. The theoretical views as to the nature of the female flower held by Celakovsky, Schumann, Strasburger, and Eichler are discussed, and a brief account of fossil Taxaceae put forward.

The author's careful and clear description of the floral structure of these plants (including that of a new genus *Acmopyle*), will be found to be especially valuable, for never before, we think, have the members of this strange group been treated in such a co-ordinated, scientific fashion, nor arranged in such an accessible whole. To take an example in detail: the way in which the natural sequence of forms is traced beteen the imperfectly developed outer envelope (here termed the "*epimatium*") of the ovule in *Microcachrys* to its perfected equivalent in *Podocarpus*, by means of the stages exhibited by different species of *Dacrydium*, is very interesting. It is merely in parenthesis we add that in the author's views on the morphology of the parts described we do not share.

The body of the work is composed of Latin diagnoses of the Genera, descriptions of all known species being given, while after each generic heading a useful key to the species finds its place.

The illustrations form one of the admirable features of the book, in which for the first time a wealth of clearly and accurately-executed detail has been collected together for our study. We may mention, *e.g.*, that no fewer than twenty-two species of *Podocarpus* are represented in the figures. Many of the drawings are original, while others are reproductions from well-known sources.

W.C.W.

HETEROTYPE KARYOKINESIS AND ITS SIGNIFICANCE.

AT the meeting of the Royal Society held on December 10th last, Professor Farmer presented some of the results of work carried out by him, in conjunction with Mr. J. E. S. Moore and Mr. C. E. Walker, on certain points in the cytology of malignant tumours (or what are known in common talk as "cancer"). The main discovery involved has the widest bearings, and if well-founded (as there is every reason to believe) will, we may hope, form the starting-point of new lines of progress in biological science. It is in fact one of the most important discoveries that has been made since the modern study of cytology came into existence not much more than thirty years ago, and forms a kind of turning-point, at once completing the series of leading facts concerning nuclear division which we owe to the distinguished cytologists of the last three decades, and opening a fresh chapter in the history of cyto-physiological knowledge. We make no apology, therefore, for the present notice, though the work in question is not strictly botanical.

The main facts of "heterotype" karyokinesis will be within the knowledge of all botanists who have followed the recent progress of cytological research; but without going into the history of the subject it may not be out of place to recapitulate them briefly here The essential points distinguishing the heterotype form of nuclear division from the ordinary homotype (somatic) form occurring in the cells of the bodies of all animals and plants may be given under four heads:—

(i.) The cell in which the heterotype division is going to occur passes through a phase of rest and growth accompanied by certain changes in the chromatin of the nucleus.

(ii.) The chromosomes, or rod-like bodies into which the chromatin net-work of the nucleus is resolved at the onset of nuclear division appear in half the number characteristic of all the preceding somatic divisions.

(iii.) The chromosomes are strikingly different in shape from those seen in the somatic divisions. Instead of having the form of V-shaped rods, they are seen as loops or rings or various modifications, and lie longitudinally on the threads of the achromatic spindle.

(iv.) The division of the chromosomes, which in somatic karyokinesis takes place by means of a longitudinal splitting, apparently always occurs, in the heterotype, by means of a transverse breaking of each chromosome. There is considerable difficulty in the interpretation of the appearances seen at the actual splitting of the heterotype chromosomes, and the authorities are not entirely agreed as to the occurrence of transverse breaking in all cases, but the mode of splitting is certainly always very different from that occurring in the homotype, and its universal interpretation as a transverse breaking appears to be at least a possible view.

Heterotype karyokinesis has been hitherto known only in the cell-divisions preparatory to the formation of the sexual cells in animals and in those plants which have no "alternations of generations," (*e.g.* the seaweed, *Fucus*), and in formation of the spores, *i.e.* the cells which give rise to the sexual generations (gametophyte) in those plants which have alternation of generations.

The discovery which we now owe to Professor Farmer and his collaborators is that of the occurrence of the heterotype in the dividing cells of malignant growths in the human body.

In typical "epithelioma" the invading cancerous tissue shews a cycle of ordinary somatic divisions. Later on the cells become rounded off losing the "prickly" appearance due to the continuity of the protoplasm at certain points between adjacent cells, and assuming the appearance of embryonic-tissue, well-known as characteristic of malignant tumours. In a varying number of rather large cells often situated in an ill-defined zone behind the growing edge of the advancing "neoplasm" (as the cancerous growth is called) the nucleus was seen to have grown to a considerable size. The prophase of division in these cells shewed a typical heterotype, the chromosomes being of the characteristic form and obviously shewing a reduced number, which could often be determined as about half of that shewn in preceding divisions. In subsequent divisions the cells of course return to the homotype. Other types of malignant growths shew similar phenomena, but the heterotype is entirely absent in the so-called "benignant" tumours.

Since the heterotype is, so far as we know, apart from malignant tumours, only found at the formation of gametes (sexual cells) or the gametophyte (sexual generation) it is proposed to call the neoplasms in which it occurs *gametoid tissues* in order to mark their similarity in this respect with gamete-forming tissues. It is important to notice that gametoid tissue, though it may be superficially similar, has no essential resemblance to true embryonic tissue which always has double the number of chromosomes, and shews typical homotype divisions.

This fundamental cytological resemblance between the tissue producing sexual cells and that of malignant tumours no doubt implies some deep-seated physiological similarity. It is suggested by Professor Farmer and his collaborators that the immediate cause of malignancy is the transformation of the type of cell-development represented by the change from homotype to heterotype, while the remote cause, though at present obscure, must lie in the action of physiological stimuli, perhaps of the nature of continuous irritation, which, in some cases at least is known to be connected with the origin of cancer. The normal cycle of development in the life of an organism is probably determined by a definite succession of automatically produced physiological stimuli, and this succession is interrupted, so to speak, at two points in the life-cycle, corresponding with the reduction of chromosomes and with the process of fertilisation (at which the number of chromosomes is doubled) respectively. Each of these interruptions forms a new starting point and is succeeded by a new development, corresponding no doubt with the inauguration of a fresh succession of stimuli. It is now probable that the new departure occurring in a malignant tumour is of fundamentally the same nature as that which marks the origin of the sexual cells or of the gametophyte in plants. It is significant, as the authors point out, that in the higher plants the gametophyte is more or less parasitic upon the parent organism, just as the malignant tumour is. It must however be remembered that in the Archegoniates it is the sporophyte, the product of fertilisation which is parasitic on the gametophyte, as the embryo of many animals is on the parent. We may therefore, perhaps, take the more general ground that the change in the course of development, the inauguration of the new series of automatic stimuli, both at chromosome-reduction and at fertilisation, is equivalent to the establishment of independence in the new organism which may lead to a parasitism on the preceding generation, more or less controlled, in the case of the normal life-cycle by the necessity of adaptation to the continuation of the species, but in the case of malignant growths, of course, quite uncontrolled in such a way.

We have in the present notice, dwelt wholly on the general biological aspects of the new discovery. With its pathological, clinical and possibly therapeutic implications we cannot, of course, deal. It seems however to negative the theories of a specific organism as the cause of the growth of malignant tumours, unless indeed we suppose the action of such an organism to be itseelf the cause of the heterotype. The discovery certainly provides a definite means of diagnosis of malignant as opposed to harmless tumours. And, above all, it not only forms a secure starting point for future investigations on the nature of cancer, but also, we may hope, will help to throw light on the more intimate nature of the series of events making up the fundamental features of the life-cycle of all but the very simplest organisms.

A NEW PTERIDOSPERM.

IN a recent number of this Journal (Vol. II., p. 73) attention was drawn to the attribution of one of the fossil seeds belonging to the genus *Lagenostoma* to the well-known Cycadofilix *Lyginodendron.*

Now, after an interval of only a few months, we have to record the discovery by Mr. R. Kidston, F.R.S.,[1] of three specimens of fragments of fronds of *Neuropteris heterophylla*, Brongn., each bearing a large seed. The specimens are preserved as impressions, not petrifactions, and differ in this respect from those which led to the recognition of *Lagenostoma* as the seed of *Lyginodendron.*

Some years ago Mr. Kidston described what he now recognises as the pollen-bearing organs of *Neuropteris heterophylla*, hence we possess unusually complete information as regards the habit of the reproductive organs of this plant.

As *N. heterophylla* is without doubt the foliage of a *Medullosa*, we thus have two families, the Lyginodendreae and Medulloseae, each known to contain a well authenticated seed-bearing representative. In this connection it may not be without interest to note that the proposal has been made[2] "to found a distinct class, under the name *Pteridospermæ*, to embrace those Palæozoic plants with the habit, and much of the internal organisation of Ferns, which were reproduced by means of seeds. At present, the families Lyginodendreæ and Medulloseæ may be placed, with little risk of error, in the new class, Pteridospermæ." O.

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[1] On the Fructification of *Neuropteris heterophylla*, Brongn. Read at the Royal Society, December 3rd, 1903.

[2] Scott and Oliver. On the Structure of the Palæozoic Seed, *Lagenostoma Lomaxi*, with a Statement of the Evidence upon which it is referred to *Lyginodendron*. Read at the Royal Society, January 21st, 1904.

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RESIDUAL VITALITY:

AN OUTLINE SKETCH OF OUR KNOWLEDGE OF THE PHYSIOLOGY OF THE SUB-NORMAL CELL.

BY F. F. BLACKMAN.

EVER since 1828 when Wohler broke through the boundary between inorganic and organic substances by preparing artificially, from its elements, the first complex organic substance (urea) identical with one of the products of protoplasmic activity, the chemist has progressed steadily towards the goal of depriving the living cell of all chemical monopoly. Already, after the capture of numerous outworks, the first line of protoplasmic defence has been carried by the synthesis of sugars of all kinds in the laboratory, and strenuous attack is being made on the second line—the synthetic production of proteids. When however there is any question of preparing substances on the manufacturing scale or at a minimum cost of production, the cell, more especially the green plant-cell, which is the only real producer among the cells of vegetables and animals, leaves the laboratory inevitably far behind owing to its one great natural resource—the power of utilising directly the energy of the sun to work up carbondioxide, the great waste product of all other organic activities.

In addition to this synthetic attack by chemists intolerant of any monopoly of production of any substance whatever by the living cell, there has developed in recent years an analytic attack, the work of biologists, the aim of which is to break up that aggregate of chemical machinery, the cell, into its constituent machines and to obtain these isolated as far as possible and to compel them to do their work under simple and controlled environments determined by the experimenter.

In this line of work the difficulty of success increases with the complexity of the part-machinery that it is sought to isolate from the general control of the cell. The enzymes we have overcome without a very strenuous struggle, and, isolated one by one with the progress of knowledge, most of them can now be preserved and at any time can be made to exhibit their respective fermentations under conditions (as regard temperature, concentration and presence of the products of their activity) quite comparable with those holding within the living cell.

The "alcoholic" fermentation process, in which sugar is split up with the production of alcohol and CO_2, remained for a long time apart, until in 1896 the researches of Buchner showed that it could be carried on abundantly, in a test tube, with the juice expressed from yeast cells disintegrated by grinding with a sharp powder, while kept very cold throughout.

The existence of a specific enzyme "zymase" that somehow is normally destroyed when the whole yeast cell dies, and is under any condition not very permanent, was the simplest hypothesis in explanation. The researches of Macfadyen in 1900, however, make us hesitate before assuming that we have here the action of so small a detached fragment of the cell-machinery as an enzyme. He points out that this expressed juice uses up more sugar than reappears in the form of alcohol and CO_2, and that this extra amount of sugar disappears completely and no trace of it in carbohydrate-form can be found, either by hydrolysis or by other means. There is apparently still existing in this expressed juice a sufficiently complex part of the cell-protoplasmic machinery to be capable of building the sugar irremoveably up into its own substance by anabolic process. Now this indicates a very high organisation still undestroyed, and possibly sufficient of the chemical manifestations of vital protoplasmic activity are present to justify calling this viscous juice "expressed protoplasm," and its action residual vital activity. If so the alcoholic fermentation is possibly due to a portion of this machinery of greater complexity than the ordinary enzyme.

The most ambitious attack that has yet been made upon those functions which have been authoritatively held to be the prerogative of protoplasm is to be found in the recent work on the question whether the photo-synthesis of CO_2 can be carried out independently of vitality as a more or less isolated phenomenon *in vitro*.

This is a question of the first importance. Should it ever be possible to control such a reaction there will open up to the imagination the prospect, remote enough indeed, of the laboratory competing with the green plant in the direct utilisation of the sun's energy for the synthetic production of substances suitable for the food of man.

Before considering the present state of experimental work on the functions exhibited in states of reduced vitality, a few words on the theoretical aspect of the subject may be desirable.

The functions of a plant-cell are justifiably to be regarded as a series, passing from simpler to more complex, the possibility of carrying on the latter depending upon the efficiency of the former subordinate ones. In most modes of death all these are not destroyed at once, and speaking quite broadly, they cease in order from above downwards. The phenomena of irritability are lost before the metabolic machinery fails and even the casual application of narcotic and other drugs will serve to throw the former out of action and leave the latter unimpaired. Growth too by the same two tests is found to be a function superposed on such metabolic processes as suffice for merely continued healthy existence. The organisation of the primordial utricle which enables it to act as a semi-permeable membrane and remain turgid, is lost (during slow death by starvation) at a point previous to the rapid collapse of the respiratory function[1]. Assimilation again may be entirely destroyed by the prolonged action of an injuriously high temperature before the respiration is at all prejudicially affected[2]. The enzymic functions of a cell often persist after respiration and all higher functions have ceased.

In such a prolonged moriturient sequence no point can be fixed as an absolute death-point for the cell. The loss of the power of keeping turgid (which can be conveniently measured by the corresponding incapacity for being plasmolysed) furnishes an obvious conventional point, from beyond which there can presumably be no return, at all events for the cells of the higher plants.

[1] The writer kept a cut-off *Tropaeolum* leaf for ten days in the dark without water but in an atmosphere saturated with moisture and estimations of the weight of the leaf and its CO_2 production were made at frequent intervals. For about nine days there was no serious loss of weight and then suddenly the organisation of the primordial utricles failed and the cell sap escaped into the intercellular spaces and out through the stomata, leading to a rapid loss of weight. Soon after this the respiration which previously was fairly high came down with a run towards zero.

[2] The writer has found that if a single leaf of Cherry Laurel be kept at a temperature of 35°C in the light and supplied with CO_2, after about 36 hours its assimilation suddenly begins to fail and diminishes to zero leaving the leaf in a condition in which its respiration is still normal and it produces as much CO_2 in the light as it did before in the dark.

The laboratory methods that have been most used to bring about the condition of reduced vitality with accompanying cessation of more or fewer of the higher cell-functions are drought, heat, mechanical disintegration and drugs.

The complete dessication of a vegetative cell of a higher plant generally produces complete death, a cessation of all the vital functions with no possibility of recovery on wetting again. With the lower plants (many Algæ and some Mosses and Liverworts) complete recovery of all functions may take place quickly on wetting after prolonged dessication and such also is the case with the specialised propagative parts of higher plants (spores and seeds). Between these two extremes there are undoubtedly intermediate cases in which there is a recovery of the lower cell-functions and not of the higher, cases for example in which there is recovery of the functions of respiration and turgidity without recovery of the assimilatory function. Considering the matter from an evolutionary standpoint it would appear to be normal for a cell to be uninjured by drying up. When the effect is death we may attribute this to some secondary cause.

As regards the effect of heat we must carefully distinguish between the effect of heating cells with high and with low water content. Air-dry seeds (with their 10 °/ₒ of water) will stand a much higher temperature than the same seeds when once wetted. Seeds when completely freed from every trace of water will survive heating to such high temperatures as 120°C as Just showed in 1877. The explanation of this lies in the fact that dry proteids are not coagulated by heating to 100°C; and Farmer has shown that the less the percentage of water contained in solid egg-albumen the higher it must be heated to render it subsequently insoluble.

This resistance to heat when dried probably holds for some of the vegetative cells which can withstand dessication: one may not therefore assume in a case in which, for example, a dried leaf after being heated, still retains the power of liberating oxygen in the light when wetted, that this phenomenon is due to nothing more complex than an enzyme. It may possibly be due to residual protoplasmic activity of a higher order. The bearing of these considerations on recent controversy will be apparent presently.

Mechanical disintegration was first successfully used by Buchner in his work on yeast. The effect varies with the completeness of the procedure and the nature of the plant-material. The writer has found that if wheat be ground up finely and then wetted it produces abundant CO_2 and respires for some hours just as if it were germinating normally. On the other hand disintegration is at once fatal to the phosphorescence of luminous bacteria, a phenomenon dependent on the presence of oxygen. Macfadyen showed this in an ingenious way. He found that this *Bacterium*, like most others is quite uninjured by extreme cold even by being dipped straight into liquid hydrogen though at such a temperature it of course ceases to be luminous. On being removed from the liquid it at once begins to glow again if whole, but if it is mechanically disintegrated at the temperature of liquid hydrogen then on withdrawal it never recovers its luminosity.

Friedel was the first, in June 1901, to assert the extraction from leaf-cells of spinach, of a substance which was he proposed to regard as an enzyme, capable in the presence of suspended chlorophyll and in the light, of decomposing CO_2 and producing oxygen. The enzyme is prepared by extracting with glycerin, precipitating with alcohol and dissolving the flocculent precipitate in water. The chlorophyll powder is obtained by drying spinach leaves at 100°C and powdering them. Friedel states that neither of these substances produces oxygen without the other and that the mixture only gives positive results in the summer. Harroy and Herzog working in a similar way state that they could not obtain any positive results even in summer.

Macchiati asserts that he obtains positive results, often of considerable magnitude, but is opposed to Friedel for he holds that the leaf-powder dried at 100°C will work freely when suspended in water, giving off bubbles of gas which are collected in a tube and analysed. Both authors fail to state the absolute amounts of oxygen which they obtained and generally speaking the presentation of the facts is confused and unsatisfactory.

About the same time as Friedel, Beyerinck independently approached the subject in another way. Instead of gasometric methods he used the extraordinarily sensitive test for oxygen which is provided by phosphorescent bacteria. Having pounded up fresh leaves in a mortar with some water, he filtered the extract into a tube, filled it up with a *bouillon* culture of the *Bacterium* and sealed it hermetically. The *Bacterium* glows until the oxygen in solution is used up and then all is dark. If the tube is placed in the sun for a minute, enough oxygen is formed, by photo-synthesis by what Beyerinck considered to be "dissolved protoplasm" and suspended chlorophyll, to keep the *Bacterium* phosphorescent for several subsequent minutes in the dark. The method is said to be sensitive enough to show the oxygen produced by illuminating with a single match.

The photo-synthetic power of this leaf-extract lasts for some hours and then begins to fail, which led Beyerinck to hold that the co-operation of the residual activity of protoplasm (dissolved!) is involved in the photo-synthesis. The most recent paper on the subject is by Molisch who confirms Beyerinck's results, but points out that his filtered leaf-extract contains complete chloroplasts and solid pieces of cytoplasm, etc. to which the photo-synthesis may be due. Ewart had already shown that isolated chloroplasts photosynthesise and so attract the oxygen-loving bacteria of the Englemann method. Molisch finds that the leaf-extract loses its power immediately on boiling, but that it can be evaporated at ordinary temperatures and kept dry for a few days with only a partial diminution of its activity.

From leaves dried at a temperature of 35°C an active extract too is obtained but not so from leaves dried at 100°C. All extracts lose their power when passed through a fine porcelain filter which removes all solid particles whatever.

Lamium album was found to give the most potent extract, while with many plants some secondary cause destroys the activity in the process of extraction. In trying to sum up the researches on this particular point we are brought up by a mass of divergent statements so contradictory as to be, on any hypothesis, rather discreditable to our subject.

The work of Molisch carries with it the greatest conviction and leads one to conclude that photo-synthesis can exist to a small degree apart from the living cell. One may further hazard the hypothesis that this function is correlated with some machinery more complex than an enzyme but much less complex than a complete protoplasmic unit.

A hopeful direction for seeking further light on the problem should be by way of determining whether an active extract can be obtained from cells which have been robbed of their power of assimilation by such special treatment as the leaf mentioned in the previous footnote.

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SUCCULENT LEAVES IN THE WALL-FLOWER (*CHEIRANTHUS CHEIRI*, L.).

BY L. A. BOODLE.

[WITH FIG. 62.]

AMONG the characters of maritime plants, a common one is the possession of succulent leaves. This is to be found among species which only grow near the sea, and also in numerous maritime forms or varieties of inland species.

Lesage[1] investigated a considerable number of plants and found that for the majority of them the leaves were thicker in specimens from a maritime situation than they were in plants living inland. His results on the structure of the leaves were as follows. In a plant growing by the sea, as compared with the inland form of the same species, if the thickness of the leaf is much increased, the palisade-cells are greatly elongated, the intercellular spaces are reduced and the chlorophyll tends to be less abundant. Lesage further determined that fleshiness, increased development of palisade, reduction of intercellular spaces and diminution of chlorophyll may be brought about in cultural experiments by watering plants (inland forms) with diluted sea-water or a solution of sodium chloride or by adding salt to the soil.[2] He subsequently proved by chemical tests in the case of specimens of two species, which had been watered

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[1] Lesage, Influence du bord de la mer sur la structure des feuilles, Comptes rendus, tome 109, p. 204; Recherches experim. sur les modific. des feuilles chez les plantes maritimes, Revue Générale de Bot., II., 1890, p. 55, etc.

[2] Schimper (Die indo-malayische Strand flora, p. 19) and others have obtained similar results.

with a solution of sodium chloride, that this salt had been absorbed by the plants.[1]

Having seen specimens of the wall-flower with some of their leaves strongly succulent in a garden close to the sea,[2] I made some experiments in order to determine whether the same character could be induced in this species by treatment with sea-salt as in Lesage's experiments.

A number of plants of the garden Wall-flower were grown in a bed at Kew[3] during the year 1903. Both first and second years' plants were used, and four of each were treated with salt, the rest of the plants being reserved for comparison. The method used was that of spraying the plants with a solution of Tidman's sea-salt of normal strength,[4] and in such a manner that nearly all the spray was expended on the leaves and very little reached the soil. This treatment was begun on June 22nd and was repeated every few days until August 6th (the average interval being four-and-a-half days). One of the seedlings died, but two of the remaining three[5] and all of the four older plants produced a certain number of strongly succulent leaves in a fairly short time, while the control plants produced none. The change to the succulent character sometimes began at the tip of the leaf, but the leaves finally became thickened throughout their length, and this often occurred in leaves which appeared mature before the treatment was begun. A week was the shortest space of time in which distinct thickening was seen to take place. The succulent leaves agree closely in external appearance and in structure with those belonging to the Wall-flowers near the sea. They are brittle, yellowish-green internally, often strongly curved (*viz:* gutter-shaped with the

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[1] Lesage, Le chlorure de sodium dans les plantes, Comptes rendus, tome 114, p. 143.

[2] I am obliged to my sister, Miss Edith Boodle, for having drawn my attention to these plants.

[3] I am indebted to the Staff of the Royal Botanic Gardens for the necessary facilities.

[4] Which is probably of about the same strength as sea water. This did not appear to injure the leaves or the growth of the plant, except on one or two occasions, when the tips of the younger leaves were "burnt" by the salt. Coupin (Sur la toxicité du chlorure de sodium et de l'eau de mer à l'égard des végétaux, Revue Générale de Bot., X., 1898, p. 177) found that, for plants germinated in the manner of water-cultures in water containing sodium chloride, a toxic effect was reached when the concentration amounted to about 1.5°/₀ for inland-plants (5 genera tried), about 3—4°/₀ for maritime plants (3 genera tried). The concentration of this salt in the Mediterranean is about 2.9°/₀.

[5] The other seedling will be referred to separately below.

concavity upwards), and may be as much as four times as thick as normal leaves. Fig. 62 shows the proportional dimensions of the succulent and normal leaves in transverse section.

Fig. 62.

Transverse sections of leaves of wall-flower treated with nitrate of silver and blackened by exposure to light. A, resistant leaf; B, succulent leaf: from photographs.

The structural features agree with Lesage's description. The palisade-cells, which normally form several layers and reach from the upper epidermis to the vascular bundles, have become very much elongated, the remainder of the mesophyll (between the bundles and the lower epidermis), which is normally lacunar and mostly of the nature of spongy parenchyma, may sometimes become largely converted into palisade-tissue, the intercellular spaces being then very much reduced.[1] This change appears to be due chiefly to increase in size of the cells, with little or no cell-division. The chlorophyll-grains also become reduced in size,[2] as was observed by Lesage in other plants, even in wild maritime forms, *e.g.* that of *Thesium humifusum* (Revue Gén. de bot., II., p. 106).

One of the four seedling plants (the one omitted in the above description) appeared for a long time to be entirely unaffected by the treatment with salt, but, by the time the experiment ended, some of its lower leaves had become somewhat thickened and brittle. Its resistant nature was probably in some way connected with its differing varietally from the other plants experimented on. It agreed with two or three of the control seedlings, but differed from all the other plants in having smaller, darker green leaves and a more compact habit.

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[1] Thus here and there practically the whole thickness of the mesophyll may be composed of palisade-tissue.

[2] To some extent this may be regarded as a pathological phenomenon, but apparently it is no more so than in the garden Wall-flower near the sea. The diminution of starch-formation in salted and maritime plants (Lesage, Comptes rendus, CXII., p. 672) need not be specially referred to here.

Salt or its component elements[1] was found to be present in considerable quantity in all the succulent leaves of the wall-flower, as will be described below.

From the method of application of the salt, it seems most likely that the salt has entered the plant chiefly through the leaves, and not through the roots. Of the small amount, falling on the soil at each spraying operation, only a small proportion would reach the roots.

Lesage noted that watering plants with salt was more effective in producing succulence than adding salt to the soil. He appears to assume that in both cases the salt enters the plant through the roots; but, assuming that the leaves were wetted in watering the plants, his observation would fit in well with the assumption of absorption by the leaves.

Though in most land-plants absorption of water by leaves is probably almost negligeable in proportion to absorption by roots, it appears that the leaf, provided it can be wetted, is slightly permeable to water (with dissolved salts).[2] To test the permeability of the epidermis in the Wall-flower, leaves attached to the growing plant were bent down into beakers containing a watery solution of saffranine, and it was found that after three-and-half hours the stain had penetrated at a few points to a considerable depth in the mesophyll. The penetration was greater in the succulent than in the normal leaf, and in the former the point of entry was often the base of a hair, which had been thrown off.

For comparison with the method of spraying the leaves, another experiment was made. The soil around one of the original control-seedlings was heavily watered with salt-solution of the same strength as that used for spraying. This was first done July 17th, and was repeated every time the other plants were sprayed, but after three-and-a-half weeks no succulent leaves were to be seen. As only one plant was treated in this way, and as one cannot tell

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[1] According to Brick (Biol. u. vergleich. Anat. d. baltischen Strandpflanzen, Schriften d. naturforsch. Gesellsch. in Danzig, Neue Folge, Bd. VII., Heft. 1, Ref. in Bot. Centralbl., Bd. 39, 1889, p. 37), and Diels (Stoffwechsel u. Struktur der Halophyten, Pringsheim Jahrb., Bd. 32, 1898), the sodium becomes combined with an organic acid in the cell-sap in halophytes.

[2] Pfeffer. Physiology of Plants, English Edition, p. 160. Dandeno (Transactions of the Canadian Institute, Vol. VII., Part 2, 1902) quotes the literature relating to the absorption of water and salts by leaves, and describes his own experiments on the subject.

what amount of salt reached the roots, it is not advisable to draw any conclusions from this experiment.

In the leaves of the different plants experimented on, the presence of chlorine and of sodium was tested by nitrate of silver and by a flame-reaction, respectively. Watery extracts (obtained by boiling) from equal weights of the normal leaf (not sprayed), the leaf of the resistant plant (sprayed) and the succulent leaf (sprayed) were compared. On addition of nitrate of silver practically no precipitate was given by the extract from the normal leaf (A), but fairly heavy and about equal deposits of chloride were yielded by the resistant (B) and the succulent (C) leaves. A similar result was obtained by cutting sections of fresh leaves and mounting them in a solution of nitrate of silver, the precipitate in B and C being fairly uniformly distributed throughout the mesophyll and epidermis.

In the case of the sodium-flame reaction (obtained by burning part of a leaf and holding the ash in a bunsen-flame[1]) the result was as follows. The normal leaf showed only slight sodium-contents, the other leaves very considerable amounts, the succulent leaf giving a decidedly brighter flame than the resistant leaf. The succulent leaf need not, however, contain more sodium than the resistant leaf per unit of weight, though it probably does per unit of length.[2]

The experiments made last year on the Wall-flower were of a rough preliminary nature, but I hope later to carry out more precise and extensive experiments.

The results so far obtained show that in this plant succulence is readily induced by the action of sea-salt, and one may fairly safely assume that, in the experiments, the salt was chiefly absorbed through the leaves. The further result is obtained that, in a plant differing only slightly from the typical form, a large amount of salt may find its way into the leaves without bringing about this effect to the same extent, or, at any rate, so quickly as in the normal form.

The cultivated Wall-flower is an instructive plant for experiments under varied treatment, for, besides growing under its normal mesophytic conditions, and enduring severe treatment with salt, it can also grow as a xerophyte, *e.g.* on walls, where it has no soil other than the mortar.

We may now refer to the structure of the leaves of Wall-flowers

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[1] This was also examined spectroscopically.

[2] The plant salted through the soil gave a fairly bright sodium-flame, but less so than the resistant plant.

growing on walls. The leaves vary in appearance, possibly in relation to the average dampness of the wall among other factors. They are usually more upright than in plants grown in soil, often small and distinctly succulent (*e.g.* as much as three times as thick as a normal leaf and slightly brittle), and in that case show great elongation of the palisade-cells, as in plants treated with salt. They usually show less reduction of intercellular spaces than in the latter, but greater thickening of the outer wall of the upper epidermis ($10{\cdot}3\mu$ was the greatest thickness noted).

From the foregoing it is seen that under xerophytic conditions and under halophytic conditions (natural or artificial) the Wallflower tends to become succulent, the structure being, on the whole, similar in the two cases.[1]

The xerophytic character of halophytes has been pointed out by Schimper and others, and also the fact that several examples are known of one and the same species occurring as a halophyte and as a xerophyte (by the sea and in Alpine habitats, but not in intermediate situations), as is the case with *Plantago maritima*.[2] The structure of a halophyte or a xerophyte, as the case may be, is often suitable for existence in the alternative situation. Warming[3] points out that many plants usually classed as halophytes should be regarded as being simultaneously halophytes and xerophytes, *e.g.* those growing on sand-dunes close to the sea.

In view of this fact, Warming, while admitting the resemblance between the two classes of plants, has attempted to find whether there are any points of structural difference between true halophytes and true xerophytes. He finds, in the halophytes he dealt with, that on an average the outer wall of the epidermis is not specially thick,[4] while it is eminently so in xerophytes; he also thinks that a point of difference is to be found in the nature of the many-layered palisade-tissue. Where the cells are much elongated, it is the outer

[1] The palisade on the upper side of the leaf usually consists of more numerous layers in plants from a wall and from near the sea than in an artificially salted plant.

[2] Schimper, loc. cit., p. 28.

[3] Warming, Halofyt-Studier, D. Kgl. Danske Vidensk. Selsk. Skr., 6 Rœkke, naturvidensk. og mathem.. Afd. VIII., 4, pp. 235, 239, 243. I am indebted to Mr. W. C. Worsdell, F.L.S. for kindly lending me the manuscript of a translation of this work, which he has made.

[4] Several plants, however, from the Mangrove-formation figured by Schimper (loc. cit., Taf. IV.) have a very thick outer wall.

ones that are most affected in xerophytes, the inner in halophytes.[1] Now in the Wall-flower it is a common occurrence for the inner palisade-cells to be the longest, both in the leaf made succulent by salt and in the leaf of a xerophytic plant from a wall.

Thus this plant when growing as a xerophyte forms an exception to Warming's generalisation. Assuming that this rule is of fairly wide application, a possible explanation of the exceptional structure of this plant may be suggested. The wild form of the Wall-flower may have *originally* been a halophyte, and the garden-form when grown under xerophytic conditions[2] may revert to halophytic structure, as being the special type of xerophytic structure that these conditions might be expected to induce in such a plant.[3] I have not so far been able to examine the structure of the leaves of the wild form. In natural habitats it grows on rocks (sometimes near the sea), so the halophytic origin of the species is not excluded. In external appearance the leaves from natural habitats are sometimes similar to those produced by the cultivated form when growing on walls.

That all halophytes, even such as grow in the wet soil of the Mangrove-formation, show some kind of xerophytic character, *e.g.* succulence, reduction of transpiring surface, dense hairy covering or a coating of wax, has been explained by Schimper[4] as a protective arrangement against too great transpiration, which would

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[1] Warming (loc. cit., p. 243) makes the following suggestion: "This structure seems to indicate that turgescence is greatest in the inner part of the leaf, perhaps because the substance causing the turgescence (the salt, presumably) is chiefly carried to or comes to act upon the innermost cells." Possibly increased turgescence may be the direct cause of artificially produced succulence, as indicated by Brick (loc. cit.) and others. Succulence can be induced by other salts besides sodium chloride, *e.g.* potassium nitrate, as shown by Schimper (loc. cit., p. 21). In the succulent leaves of the Wall-flower the expansion of the mesophyll appears to have stretched the epidermis slightly, and the "hyponastic" curvature of the leaf may be due to the lower epidermis being capable of greater extension than the upper, as it has thinner walls.

[2] There is a possibility that some substance easily extracted by the plant from the mortar may produce pseudo-maritime conditions. Potassium-contents appeared to be somewhat more marked than in normal plants. It may be noted, though only having an indirect bearing on the question, that leaves of *Linaria Cymbalaria* from the same wall had a palisade, usually two cells thick the *outer* cells being the longest, where there was a difference.

[3] An interesting problem is here suggested, *viz.* whether the plasticity of a species has any relation to its past history as regards changes of habitat.

[4] Schimper, loc. cit., pp. 12, 26. etc.

lead to an injurious accumulation of salt in the plant. Other theories have been suggested or discussed by different authors, *e.g.* Stahl, Diels and Benecke.[1]

These need not be entered into here, but in view of the fact that all halophytes show xerophytic characters of some kind, it appears that these characters are either necessary to existence in a maritime situation or are the natural result of a halophilous mode of life. As one such character, namely succulence, may be directly induced by treatment with salt, it may be suggested that, in certain cases of an inland form spreading to a maritime situation, this character may have been directly produced in the first instance by the action of salt, and may afterwards have become fixed[2] by the adaptive selection of other characters (physiological and structural), which cause succulent development apart from the action of salt.

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[1] Stahl, Einige Versuche über Transpiration u. Assimilation, Botan. Zeitung, Bd. 52, Abt, 1, 1894, p. 117; Diels, Stoffwechsel u. Struktur d. Halophyten, Pringsheim Jahrb., Bd. 32 (1898); Benecke, Ueber die Diels'sche Lehre von d. Entchlorung d. Halophyten, Pringsheim Jahrb., Bd. 36, p. 179.

[2] Some succulent maritime plants retain their succulence, when cultivated without salt, others form thinner leaves under these conditions; Lesage, Revue Générale de Bot., 1890, p. 56.

THE SEEDLING-STRUCTURE OF CERTAIN PIPERACEAE.

By T. G. Hill.

THE present preliminary communication deals with the transition-region between root and stem of *Piper cornifolium*, H. B. K. and *Peperomia maculosa*, Hook. Notwithstanding the fact that these genera are closely allied, there are considerable differences in the behaviour of the structures concerned, and the transition in both plants is of a type which does not appear to have been hitherto described.

Piper cornifolium. Running longitudinally through each cotyledon there is one bundle, which, near the base of the seed-leaf, bifurcates and each half revolves in such a manner that the centrally placed xylem becomes bounded on each side by a group of phloem elements. Concurrently with these changes the protoxylem of each half-bundle gradually twists so as to occupy a dorsal position as regards the cotyledon. This rearrangement in the vascular tissues of each cotyledonary bundle is strongly marked in the base of the petiole of the seed-leaf; the completion and re-fusion being effected in the outer cortex of the axis.

The bundles on entering the stem travel directly to the centre; the opposing groups of phloem fuse together, the protoxylems are already in their proper root-position and thus a typical diarch root obtains.

The chief interest in the seedling-structure of this plant obviously lies in the fact that the twisting and rearrangement of the cotyledonary bundles takes place chiefly in the seed-leaves and thus the hypocotyl is, as regards structure, practically non-existent.

PEPEROMIA MACULOSA. A single bundle enters the axis from each cotyledon, and not only is each of these traces comparatively much smaller than the corresponding structure in *Piper*, but also no such rearrangement of the phloem and xylem, as described above for the last named plant, has been observed. A little below the level of the insertion of the seed-leaves the epicotyledonay strands fuse together, into two large collateral bundles with their protoxylem groups separated by a few parenchymatous elements. The phloem of each of the collateral cotyledon-traces fuses with the corresponding tissue of these epicotyledonary bundles and the xylem passing between, occupies a position in contact with the protoxylem of the epicotyl bundles and, as far as can be ascertained, plays no further part.

Much of the remaining portion of the main axis does not exhibit a really typical root-structure, and it is only near the apex that an appearance approximating to that of a normal root, in transverse section, occurs.

The differences between the seedling-structure of these two plants are thus of some importance, and require no further emphasis here. A complete account of the facts observed in these and other plants of the Order will appear in the Annals of Botany.

SECONDARY TRACHEIDES IN *PSILOTUM.*

By L. A. Boodle.

(Preliminary Account).

THE vegetative structure of *Psilotum* is in most respects well known from the works of Bertrand[1] and other authors.

In *Psilotum triquetrum* the plant may be roughly described as consisting of a branched[2] subterranean rhizome and of vertical aerial shoots. In the vertical shoots, which bear scale-leaves, a transverse section of the stele contains an exarch polyarch xylem-mass, which has the form of a star enclosing a strand of sclerotic tissue. Towards the base of the aerial branch this structure becomes simplified by the gradual decrease in the amount of sclerenchyma present, until none is left, and the xylem then forms a solid strand, which has previously become reduced first to tetrarch then to triarch structure. Thus the vascular system of the lower part of the aerial shoot has a typical exarch protostele. The rhizome also is protostelic, containing an exarch triangular (triarch) or often band-shaped (usually diarch) mass of xylem or a small group of tracheides (*e.g.* 5—6) with no very definite protoxylem. A certain amount of parenchyma sometimes three or more cells thick lies between the xylem and the sieve-tubes.

The above description refers to primary tissue only, and nothing more than this appears to have been hitherto observed, to judge from the descriptions of Bertrand, Russow,[3] etc.

In the lower part of the aerial shoot and in portions of rhizome directly connected with aerial shoots a certain number of tracheides were found amongst the parenchyma, which lies between the usually compact primary xylem and the ring of sieve-tubes. These tracheides generally include a few or several in course of development, these elements being incompletely lignified and sometimes containing protoplasm. Immature tracheides are to be found in parts of the plant which are probably several months old, *e.g.* at the base of a well developed aerial branch, and throughout the branch (2 cm. long), from which the former sprang, and in the parent stem of this again at 2 cm. below the point of insertion.

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[1] Bertrand, Recherches sur les Tmesipteridées, Archives botaniques du Nord de la France, I. (1881), p. 252.

[2] Both dichotomously and apparently monopodially.

[3] Bertrand, loc. cit.; Russow, Vergleich. Unters.

This late addition of tracheides outside an exarch xylem-mass must be regarded as being of a secondary nature and probably representing secondary thickening, greatly reduced. Sometimes, though not constantly, indications of radial seriation are shown by the secondary tracheides or by the parenchyma outside the primary xylem; this fact acquires some little importance when taken in connection with the late differentiation of the peripheral tracheides. The fact that the secondary tracheides had not been previously recorded may be due to varietal or individual variation in this respect, but more probably to the very tough cortex, which disposes one to examine the rhizome at a younger stage of development.

A full account will be published later in the Annals of Botany.

THE ORIGIN OF FLOWERING PLANTS.

IN Mr. Seward's letter on "The Origin of Flowering Plants" printed in the December number of the New Phytologist, the subject is referred to as one of the most fascinating of botanical problems. This must be my excuse for the present communication.

Fossil Botany cannot be expected to indicate which forms first evolved the characteristic Angiospermic embryo sac. Hence so far as this character is concerned we cannot but look with interest on the facts revealed by Drs. Lotsy and Karsten. *Gnetum* is now, I believe, generally recognized as possessing an embryo sac in which it is not difficult to conceive such an evolution may have taken place. But there is still the difficulty as to the origin of the Angiospermic flower.

In spite of the fact that most of your readers have very probably considered and discarded the idea that *Gnetum* can have anything akin to the ancestor of the Angiosperms in this respect, there is one suggestion as to the nature of its fructifications which I have never heard discussed and I should therefore be glad of any criticism on it.

The ovule of *Gnetum* consists of a nucellus which is surrounded by an envelope with six micropylar lobes and is thus radially symmetrical. The nucellus and lobed inner integument appear to me on grounds given in detail elsewhere, to be equivalent to a synangium. Adopting for the moment this view we may say that the female synangium of *Gnetum* is further surrounded by two envelopes, the inner of which probably represents the outer integument of the Angiospermic seed and the outer, as has frequently been pointed out, the carpel.

Parallel parts are present in the so-called "male flower." We find a synangium (the anther) surrounded by its envelope. This envelope appears comparable with those of the female synangium. If the latter with its envelopes is a megasporophyll, we may safely regard the former with its envelope as a microsporophyll or primitive stamen. If we now call to mind the so-called "inflorescence" of *Gnetum* we find it consists of axes on which are borne, in verticils, sterile bracts, and closely approximating to these whorls the structures which may, and often have been, interpreted as mega-, or microsporophylls respectively. If so, their position in the axils of the bracts may be entirely due to the need for protection in the young state and be correlated with a loss of the internodes next below them.

Suppose now the internodes above the fertile leaves to be likewise suppressed, and the whole spike reduced to a conical torus with the loss of the now unnecessary bracts, we should then have the structure of the central part of such an Angiospermic flower as *Liriodendron*.

Moreover, as some of the "spikes" are androgynous, with the carpels occupying the uppermost nodes, we may even obtain the ambi-sporangiate flower by the same process of reduction. In *Gnetum* we know that the so-called "female flowers" terminating an androgynous spike are sterile, but that they represent ancestrally fertile organs would be granted by most morphologists.

In *Welwitschia* the telescoping has been accomplished and we have the outlines of the Angiospermic flower for the first time laid down.

Here again the central capellary organ is sterile, but we cannot doubt that it was fertile in the ancestors of *Welwitschia*. The similarity of the stamen of the latter with the so-called "male flower" of *Gnetum* strongly corroborates this view of their homology. When we add to these considerations the similarity in the structure of the *Gnetum* and the primitive Angiospermic nucelli, especially the extent of the sporogenous tissue in both, we seem to have grounds for regarding *Gnetum* as nearer the Angiosperm than any other phylum.

In Anatomy we know that *Gnetum* has characters approximating to those of Angiosperms and that its habit (foliage. etc.) is more Angiospermic than Coniferous.

In *Drimys* and other genera allied to *Liriodendron* homoxylous secondary wood is recorded, so that each phylum seems to approximate to the other on anatomical grounds.

The theory of the sudden appearance and rapid differentiation of Angiosperms which seems to be the chief outcome so far of their geological record would compel us to expect the immediate ancestor to have attained considerable vegetative differentiation.

It is a curious fact that the distichously branching spikes of *Gnetum latifolium*, if telescoped down as suggested above, would yield the partial inflorescence chararacteristic of *Castanea*, the basis according to Eichler of that of other Amentiferae.

Is not then the hypothesis a tenable one that some race which in respect of its numerous free sporophylls, nucellar structure, embryo-sac development, and vegetative characters bore considerable resemblance to *Gnetum*, finally attained the full characters of the Angiospermic embryo-sac, and that with these a new impetus was given which has resulted in the dominance of the Angiosperm ?

M. Benson.

WHAT PART DOES COLOUR PLAY IN THE ATTRACTION OF INSECTS TO FLOWERS ?

NO conclusion relating to that most fascinating subject the natural history of the flower appears more certain than the doctrine of "flag"-function of the coloured portion (usually the corolla) of an entomophilous flower in attracting insects for the purpose of carrying out cross-pollination. It seems, indeed, impossible to understand why entomophilous flowers (*i.e.* flowers which can be shewn to be adapted for cross-pollination by insects) should in general possess conspicuous coloured corollas if these do not perform the function in question. Sprengel, the great pioneer of the study of floral natural history, clearly recognised this. "The corolla of these flowers," he says, "is either wholly inexplicable, or else it serves to enable the flowers to strike the eyes of the bees which collect their pollen, at a distance." Darwin, who more than half a century later took up and extended Sprengel's work, interpreting the extraordinary adaptations of floral structure in the light of his theory of natural selection, takes essentially the same view. "We can understand" he writes in "The Effects of Cross and Self-Fertilisation," "the considerable size and striking colours (of flowers), and in certain cases also the bright colouration of neighbouring parts, as for instance, flower-stalks and bracts. By these means are they made conspicuous to insects, according to the same principle that in those fruits which are eaten by birds there is a sharp contrast with the green leaves, so that the former may be seen and thus seeds freely distributed." In some flowers the conspicuousness is obtained even at the cost of the reproductive organs, as for instance in the ray-florets of many Compositae, in the outer flowers of *Hydrangea* and the terminal flowers of the Feather-hyacinth or *Muscari*. There is also ground for the belief—and this was Sprengel's opinion—that flowers differ from one another in colour, in correspondence with the species of insects which visit them. Hermann Müller, who for many years devoted the whole of his leisure to the study of flowers and insects, and undoubtedly did more than any other naturalist to systematise the whole subject, took it for granted that the colour of flowers serves to attract insects, and made experiments to ascertain if given species of bees were specially attracted by given colours. Lubbock made similar experiments, and both concluded that bees have special colour-preferences. Upon these results, taken together with an enormous series of observations on the relative numbers of different species of insects actually visiting flowers of different colours, Müller founded a theory of the evolution of floral colour as part of his general theory of the evolution of entomophilous flowers. Grant Allen, too, published a work on the Colours of Flowers, the conclusions of which do not differ essentially from Müller's. It was therefore with some surprise and incredulity that those who follow the progress of floral natural history received the conclusions of Professor Plateau of Ghent, published in several papers from 1895 to 1902. As the result of elaborate experiments, Professor Plateau denies that there is proof of the perception of the colours of flowers by insects, and holds that it is the scent alone by which the visitors are attracted.[1] His earlier papers were criticised at the time by Kienitz-Gerloff and also by the well-known Swiss entomologist Forel, who repeated some of Plateau's experiments and arrived at quite other conclusions. In the last number of the Beihefte zum Botanischen Centralblatt, Bd. XV., Heft. 3, Eugen Andreae publishes an able paper in which he not only shows very clearly that Plateau's conclusions are ill-founded, but also records the results of a number of experiments of his own, which distinctly advance the whole subject. Andreae's observations and experiments were made in the Botanic Gardens at Jena, under the direction of Professor Stahl, at Cadenabbia on the Lake of Como, and in Corsica, both on wild flowers and in gardens. He found, however, that the garden work was much the more satisfactory of the two, since wild flowers are seldom sufficiently closely massed to enable the observer to get decisive results.

The main result which emerges from Andreae's numerous and varied experiments is precisely the enormously greater importance of colour compared with scent, in attracting the higher anthophilous bees, such as *Bombus*, *Anthophora*, *Osmia* (especially the hive-bee *Apis mellifica*) and also butterflies at a distance. The author used various methods of contrasting the effect of these two factors. Thus he covered a bunch of flowers belonging to a species he was experimenting upon, with an inverted bell-jar, so that the flowers could be seen while their scent could not escape, while a second bunch he covered with dark paper, open at the top, thus hiding the flowers from the eyes of insects but allowing their odour to rise into the air. In other cases he worked with artificial flowers, and often obtained the most striking results in inducing insects to visit them in preference to hidden, but strongly scented, natural flowers, which latter, when exposed, they were visiting freely.

On the other hand some of the bees less highly adapted to the anthophilous habit, such as *Anthrena* and *Prosopis*, were often clearly attracted by scent at short distances, particularly in an experiment with *Reseda*—where the flowers were hidden in a gauze net—and did not appear to be guided by sight. In the case of night-flying insects, such as the crepuscular Convolvulus Hawk Moth, visiting a species of *Crinum*, and of the flies visiting a species of *Arum*, it was also shown that the odour of the flowers visited was the dominating factor, though these insects were also guided to some extent by the sense of sight.

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[1] In one of his last papers, Plateau rather modifies this conclusion and attributes a "secondary action" to vision.

Andreae concludes that of the day-flying insects, the biologically higher types, *i.e.* those with a relatively long life in the winged state, and with a long direct flight, are characterised by sharp sight and are attracted to flowers mainly by their colour, while the biologically lower forms, *i.e.* those with a short life in the winged condition and a short flight, have less power of sight and are more dependent on smell. With these also come the specially adapted night-flying insects—although they include some very highly developed Lepidoptera such as the hawk-moths—as well as the whole tribe of insects comparatively little adapted to the anthophilous habit, for instance many of the flies, and some beetles. Insects which have a running rather than a flying habit, such as many of the beetles—whether anthophilous or not, depend, for obvious reasons, almost wholly on the sense of smell for discovering their food at a distance. The author also points out that the condition of bright sunlight under which brightly coloured flowers are mainly visited by day-flying insects, are just those which are favourable for enhancing the effect of colour, while the relatively dry air is unfavourable to the diffusion of scent. On the other hand the damp sultry evenings so beloved, as all lepidopterists know, of night-flying moths, are particularly favourable to the dispersal of scent.

Of course the higher bees, notably *Apis*, are constantly found in great numbers on flowers which are not highly coloured and often have a strong scent, especially when great numbers of these are massed together. The flowers of the Lime-tree are a good example. On the other hand they very rarely visit highly conspicuous double flowers where no honey is to be obtained. The explanation of this is simply that they are highly intelligent insects, which soon discover where honey is to be obtained in large quantities, and go back to the source of it again and again. Conversely, even though attracted in the first instance, they will not return to a brightly coloured but barren flower. Andreae's observations bear out both these points, which have been somewhat neglected by Plateau and others.

We may therefore regard these most recent observations as completely confirming the traditional view of the significance of colour in attracting inseets to flowers; and as establishing its predominant importance in the case of the most highly adapted diurnal anthophilous insects.

A.G.T.

RECENT RESEARCHES ON THE SPECIALIZATION OF PARASITISM IN THE *ERYSIPHACEAE*.[1]

By Ernest S. Salmon, F.L.S.

[With Figs. 63-65.]

IN the case of certain parasitic fungi the process of becoming adapted to different species of host-plants has resulted in the evolution of "biologic forms." An instance taken from the *Erysiphaceae* will show the nature of this specialized parasitism.

The species *Erysiphe Graminis* DC. occurs on wheat, barley, oats, rye, and a number of wild grasses belonging to various genera (*Bromus*, *Poa*, *Dactylis*, *Festuca*, &c.).

We will confine ourselves for the present to the forms of the fungus on wheat and on barley. In all morphological characters—even when examined under the microscope—the form on wheat is indistinguishable from the form on barley, both in the conidial *(Oidium)* stage and in the perithecial stage. But inoculation-experiments show at once that these two forms possess distinctive and sharply defined *physiological* (or *biological*) characters.

If the asexual conidia or the sexually produced ascospores of the form of the fungus on wheat are sown on wheat and barley, infection at once results on wheat, but never on barley; and similarly with the form of the fungus on barley when sown on barley and wheat. Further, if the fungus on wheat or barley is sown on species belonging to any other genus in the circle of host-plants of *E. Graminis*, no infection results.

The forms on wheat and barley are therefore "biologic forms," since they are morphologically identical, but differ physiologically or biologically in possessing distinctive and sharply defined infection-powers.

Inoculation-experiments have shown also that the forms of *E. Graminis* in *Secale*, *Avena*, *Poa*, *Agropyrum*, *Bromus* and *Dactylis* constitute likewise "biologic forms," and that the same phenomenon is found in other species of the *Erysiphaceae*, e.g. in *E. Polygoni*,

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[1] We are able to include this article, containing a general sketch of his more recent investigations, through the courtesy of the author; Mr. Salmon's detailed paper will appear in a forthcoming number of the Beihefte zum Botanischen Centralblatt. Ed.—N.P.

DC., *E. Cichoracearum* DC., *E. Galeopsidis* DC., and *Sphaerotheca Humuli* (DC.) Burr.[1]

This phenomenon of specialized adaptive parasitism is not peculiar to the *Erysiphaceae.* It was originally discovered by Eriksson[2] in the *Uredineae*, and its existence has lately been proved in other groups of parasitic fungi.[3]

Some recent work on the specialization of parasitism in the *Erysiphaceae*, which I have carried out at the Cambridge University Botanical Laboratory, has brought to light certain facts which show the existence of complicated inter-relations between "biologic forms" and their host-plants. These facts show (1) the high degree of specialization reached in the adaptive parasitism of *E. Graminis* to the various host-species within a single genus; (2) the existence of "biologic forms" of host-plants; and (3) the existence of certain host-species which serve as "bridging species."

In the series of inoculation-experiments conidia of the forms of *E. Graminis* occurring on species of *Bromus* were used, and 1650 inoculations were made on leaves of various species of the genus.

The results of the experiments show that *a considerable number of "biologic forms" exist within the genus Bromus.* Thus the fungus occurring on *B. interruptus*, *B. "hordeaceus," B. commutatus*, *B. racemosus*, *B. velutinus*, *B. arvensis*, *B. tectorum*, and *B. arduennensis* proved in a series of comparative infection experiments, to be in each case a "biologic form" possessing distinctive infection-powers.

These facts show the high degree of specialization which the fungus has reached in fitting itself to the various species of the genus *Bromus*. They show also that each species of *Bromus* has distinctive physiological (or constitutional) characters existing concomitantly with the specific morphological characters. We are able by using the index of the reaction to the attacks of the "biologic forms" of the fungus to show the presence of *specific physiological* (*or constitutional*)

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[1] See *Neger, F. W.*, in Flora XC., 221-272 (1902); *Marchal, E.* in Comptes Rendus, CXXXV, 210-212 (1902) and CXXXVI., 1280-1281 (1903); *Salmon, E. S.*, in Beihefte z. Botan. Centralbl., XIV., 261-315, Taf. XVIII. (1903), and in Journ. of Bot., XLI., 159-165, 204-212 (1903).

[2] See *Eriksson, J.*, in Bot. Gaz. XXV. 26-38 (1898), and in Ann. Sci. Nat. VIII. sér., XIV., 1-124 (1901), and XV., 1-155 (1902); also *Fischer, E.*; Die biologischen Arten der parasit. Pilze (1903).

[3] Thus in the *Hypocreaceae (Claviceps)*, see *Stäger, R.*, in Bot. Zeit. LXI., 111-158 (1903).

characters in a plant. As a rule each species of *Bromus* shows physiological characters which hold good for all examples of the species derived from different localities. But there are exceptions to this rule, and we find that the inter-relations of the "biologic forms" of the fungus with certain of their host-plants become complicated by the existence of "biologic forms" of the host-plants. Such a case is represented in the adjacent diagram (Fig. 63).

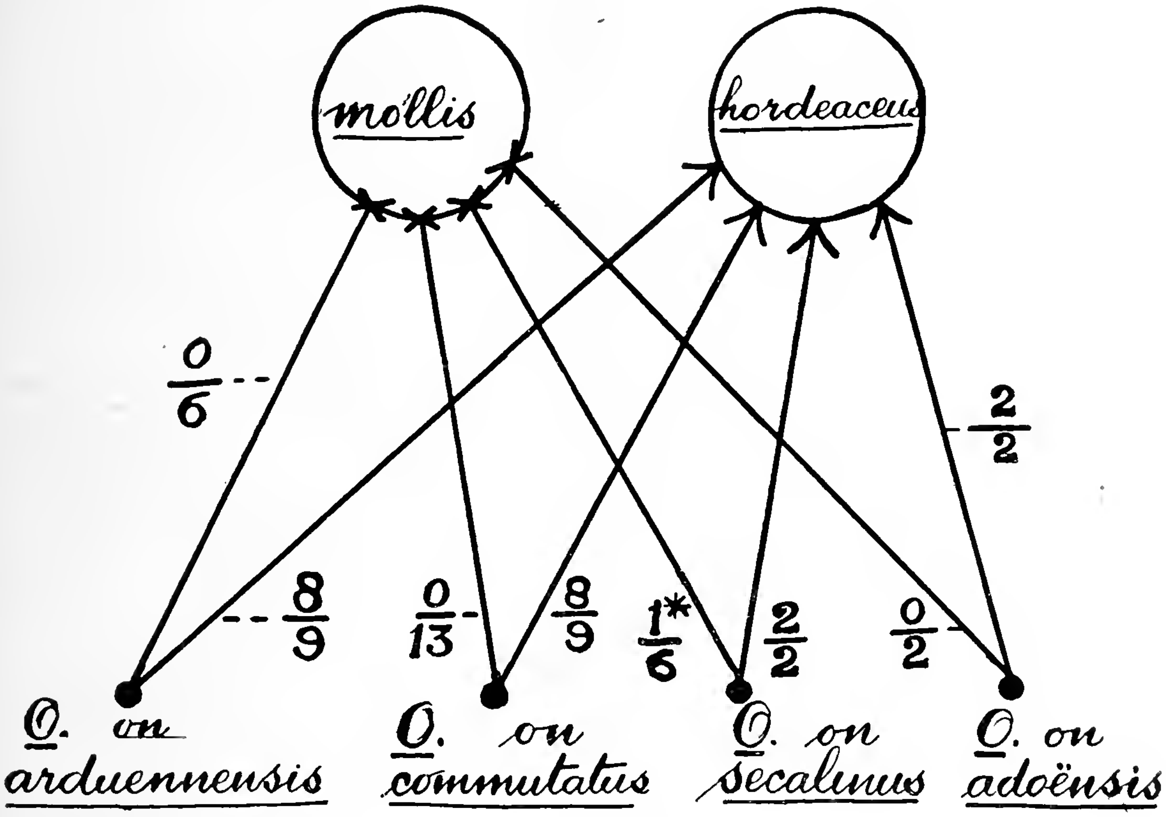

Fig. 63.

Diagram illustrating the different constitutional characters of the two plants *B. mollis* and *B. "hordeaceus"* which are morphologically identical. *B. "hordeaceus"* is infected by the four forms of *E. Graminis* on *B. arduennensis*, &c., while *B. mollis* is immune against their attacks.

Here we have represented the relations which exist between four forms of the fungus (*viz.* the *Oidium* on *B. arduennensis*, *B. commutatus*, *B. secalinus*, and *B. adoënsis)*, and the species *B. mollis* and a plant called *B. "hordeaceus."*[1] The latter plant has been grown at the Cambridge Botanic Gardens from seed originally obtained from the St. Petersburg Botanic Gardens, and is identical morphologically with *B. mollis.* Now let us observe the different

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[1] In this and in the succeeding diagrams the number of inoculations made and their results are expressed in the form of a fraction, in which the numerator expresses the number of times in which infection resulted, and the denominator the number of times a leaf was inoculated.

constitutional peculiarities shown by the two plants in the comparative inoculation experiments with the four forms of the fungus. Conidia of the fungus on *B. arduennensis* were sown six times on *B. mollis*, but no infection resulted; sown on *B.* "*hordeaceus*," infection occurred eight times out of nine. Again, conidia of the fungus on *B. commutatus* sown thirteen times on *B. mollis* produced no infection; sown on *B.* "*hordeaceus*" infection occurred eight times out of nine. Similarly with the fungus on *B. secalinus* and on *B. adoënsis*.

These results lead us to conclude that the morphological species *B. mollis* includes two "races" or sets of individuals possessing distinctive physiological (or constitutional) characters, that is to say, with regard to the forms of the fungus in question, an immune and a susceptible race. These may be termed two "biologic forms" of a host-plant.

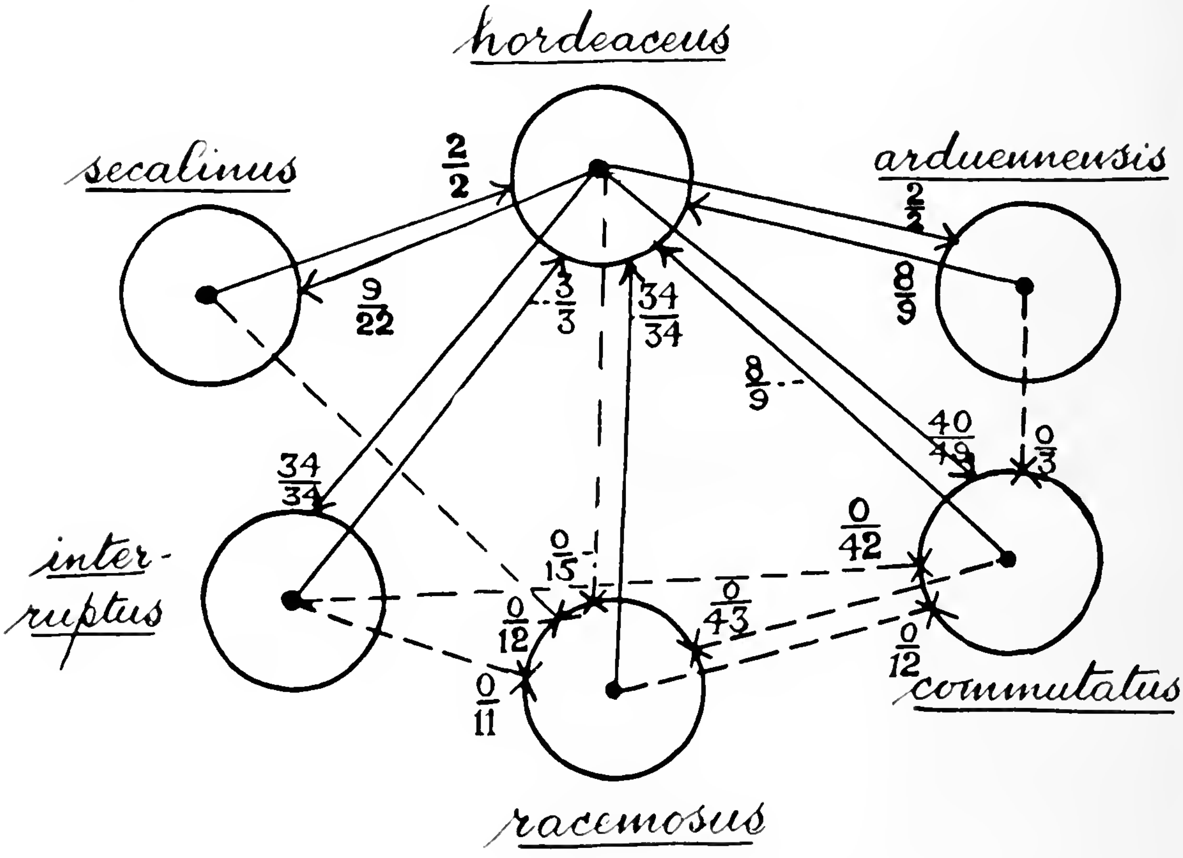

Fig. 64.

Diagram illustrating the position of *B.* "*hordeaceus*" as a "bridging species." *B.* "*hordeaceus*" is infected by the forms of *E. Graminis* on *B. racemosus*, *B. interruptus*, and *B. arduennensis*, and the fungus occurring on *B.* "*hordeaceus*" is able to infect *B. commutatus*. Assuming that the fungus produced on *B.* "*hordeaceus*" by inoculation with conidia from *B. racemosus*, *B. interruptus*, and *B. arduennensis* is able to infect *B. commutatus*, *B.* "*hordeaceus*" will serve as a "bridging species" enabling the forms of the fungus on these three host-plants to pass on to *B. commutatus*, a species which they are unable to infect directly.

There remains now to be considered the case of certain host-species which serve as "bridging species." In Fig. 64 we have represented in the first place, the relations which exist between the forms of the fungus on *B. secalinus*, *B. interruptus*, *B. racemosus*, *B. commutatus* and *B. arduennensis* and their common host-plant, *B.* "*hordeaceus*' ; secondly, the relations which exist between the fungus on *B.* "*hordeaceus*" and *B. secalinus*, *B. interruptus*, *B. racemosus*, *B. commutatus*, and *B. arduennensis ;* and, thirdly, the relations (so far as they have been ascertained) which exist between any one of the forms of the fungus infecting *B.* "*hordeaceus*" and the host-plants of the other forms of the fungus infecting *B.* "*hordeaceus*."

We see that in every case but one the fungus occurring on *B.* "*hordeaceus*" is able to infect the host-plant of the forms of the fungus which infect *B.* "*hordeaceus*." Further we see that the forms of the fungus which are able to infect *B.* "*hordeaceus*" are not capable of reciprocal infection among themselves.

With the fact of the existence of such inter-relations as these before us, the question at once suggests itself, do not such species as *B.* "*hordeaceus*" act as bridges affording a passage for certain forms of the fungus to species of host-plants which they are unable directly to infect ?

I have obtained proof that *B.* "*hordeaceus*" does in at least one case act in this way as a "bridging species."

Experiments have proved (see Fig. 64) that the fungus on *B. racemosus* is incapable of infecting *B. commutatus*, while it never fails to cause full infection on *B.* "*hordeaceus*"; and further, that the fungus found growing on *B.* "*hordeaceus*" is able to infect *B. commutatus*.

In the experiments carried out last summer I took spores of the

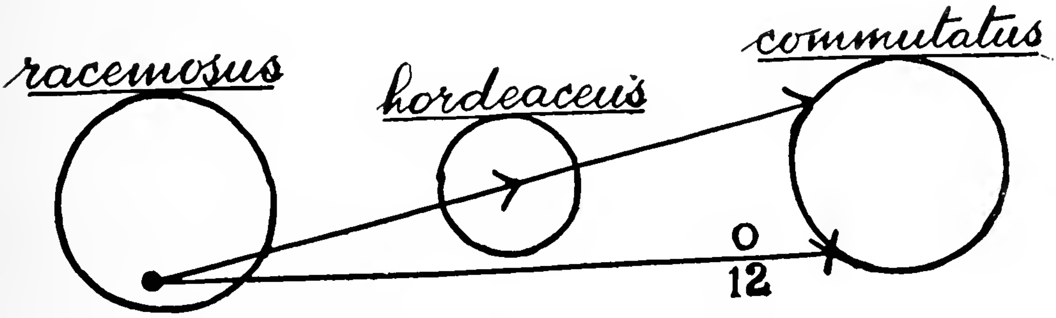

Fig 65.

Diagram illustrating the result of the experiments mentioned over-leaf, *B.* "*hordeaceus*" is here proved to serve as a "bridging species," affording the fungus on *B. racemosus* a passage to *B. commutatus*, a species which this fungus is unable to infect directly.

fungus occurring on *B. racemosus*, and inoculated with them twenty-two leaves of *B.* "*hordeaceus*." In seven days twenty of the twenty-two leaves bore patches of mycelium with conidiophores and little powdery masses of spores. These spores, produced on *B.* "*hordeaceus*" by inoculation with spores from *B. racemosus*, were sown on a leaf of *B. commutatus*, and caused full infection in eight days. This experiment, which was safeguarded by controls, gives definite proof that certain species of *Bromus* serve as "bridging species."[1] (These results are illustrated in fig. 65).

There is little doubt that when all the details of the inter-relations between the forms of the fungus and the various species of *Bromus* are worked out, the species of host which serve as meeting-places or as bridges will be found to be very numerous, and that consequently many of the "biologic forms" of the fungus within the genus *Bromus* which now appear to be distinct, will be found to merge into one another at these points.

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[1] The same kind of inter-relations between a parasite and certain hosts has been discovered by Freeman (Annals of Bot., XVI, 487-494, 1902), and Marshall Ward (Annal. mycolog. I., 132-151, 1903), in the case of one of the *Uredineae* (*Puccinia dispersa*, Erikss.) on species of *Bromus*.

* * *

R. MADLEY, PRINTER, WHITFIELD STREET, LONDON, W.

THE
NEW PHYTOLOGIST.

Vol. 3, No. 3. March 28th, 1904.

THE OPENING OF THE NEW BOTANICAL SCHOOL AT CAMBRIDGE.

On Tuesday, March 1st, the King and Queen journeyed to Cambridge to take part in the celebrations connected with the opening of the remarkable group of new buildings recently erected by the University. Whilst such different branches of study as Law, Medicine, Geology, as well as Botany, all benefit materially under the enlightened scheme now brought to a successful termination, it is more particularly with the fortunes of the last-named subject that we are immediately concerned.

The accommodation and facilities offered by the new building mark at once the outward recognition by the University of the vigorous growth of the Botanical School it has been erected to house, and the high position which that school holds in the esteem of Botanists.

How vigorous has been this growth may be shortly traced. It may be said without serious inaccuracy that the present Cambridge School has sprung into existence within the last quarter of a century. Even those whose knowledge of the Botanical Department dates back no further than twenty years, witnessed the early stages of the Botanical rejuvenescence in Cambridge. At that time the department consisted of little more than a herbarium and an inconvenient lecture room. It was Dr. Vines, by his successful and inspiring teaching, who first kindled the fire, though the facilities at his command were of the most rudimentary. For his practical classes, Dr. Vines had to be content with a small corner separated by paper screens from a room used in the service of the herbarium. This original laboratory, the germ from which the present noble building has developed, was on the ground floor at the corner on emerging from the tunnel which led from the Cavendish Laboratory to the rough northern court of the then "New Museums." For the purposes of advanced students this place was presently superseded by a nondescript gallery, on the south side of a sort of half-staircase room in which a Botanical Library was arranged. What Dr. Vines had commenced Mr. Walter Gardiner continued, and he was instrumental in the founding of the present Museum collection, in the re-organisation and reconstruction of the plant-houses at the Botanic Garden, and in the erection of the well-known "lean-to" all along the face of the herbarium to serve the purposes of a general elementary laboratory. Old Cambridge men will be grateful to Mr. Gardiner for a recently issued pamphlet, in which he details the early struggles of the Museum Department, and traces it back to the original conception of the late Professor J. S. Henslow in the remote past.

The "lean-to" was followed by the annexation of a dwelling-house, interesting from an antiquarian point of view, but, in equilibrium, leaving something to be desired as a laboratory for the pursuit of delicate physiological investigations. This annexe, however, was a pleasant spot, bathed in sunshine, and has been the centre of much good work during the last twelve or fifteen years. Throughout the whole of this early period of the revival, and continuously to the present day, the department enjoyed the very great advantage of having the support and counsel of Mr. Francis Darwin, as Deputy-Professor or as Reader—a connection that has contributed to its advancement in many ways.

With the year 1895 we come to the commencement of the later phase in the growth and consolidation of Botany at Cambridge. Professor Marshall Ward was elected to the Chair rendered vacant by the death of Professor Babington (who had long been an invalid), and to his energy and directive skill, primarily, is owing the magnificent building into which the department has just migrated. We say primarily, for of course the new building is a joint product, and represents the collective wisdom of the whole Cambridge Staff. And it is evident on inspection that nothing has been left to chance. The needs of the department from the several points of view of the general elementary student, the advanced worker, and the original investigator have been fully realised, and whilst the building is perfectly adapted to the various activities of the present *régime*, it is sufficiently elastic to admit of such adjustment as the future trend of Botanical work may dictate. As it is, the equipment of so many laboratories and rooms for physiologicaland experimental work is not only in accord with modern requirements but seems a very prudent investment for the future. The scale upon which the building has been carried out will be appreciated when it is explained that there is accommodation for 150 general elementary students, and for the higher grades in proportion. The convenience of the site, close to the other scientific departments of the University, is of course quite evident, but we cannot help thinking that the Botanic Garden would have been found in the long run an even more appropriate spot.

Externally the new school is as pleasing to the eye as its interior arrangements are acceptable to the Botanical instinct. The Architect, Mr. W. C. Marshall, whilst conceding every scientific requirement has at the same time, with rare skill, produced a dignified and beautiful building worthy of the purpose it is to fulfil.

It only remains to be said that many Botanists from all parts of the country were able to accept the hospitality of their Cambridge *confrères* and be present at the opening, and that they were delighted to see that Botany—the Cinderella of the Sciences—had at last received at the hands of the University that recognition which she has so long claimed. The very pleasant "house-warming" which followed the more formal ceremonial was the occasion for many hearty congratulations to Professor Marshall Ward and his Colleagues on the realisation of their aims, and of good wishes for the continued prosperity of the School.

O.

A PROBABLE PARASITE OF STIGMARIAN ROOTLETS.

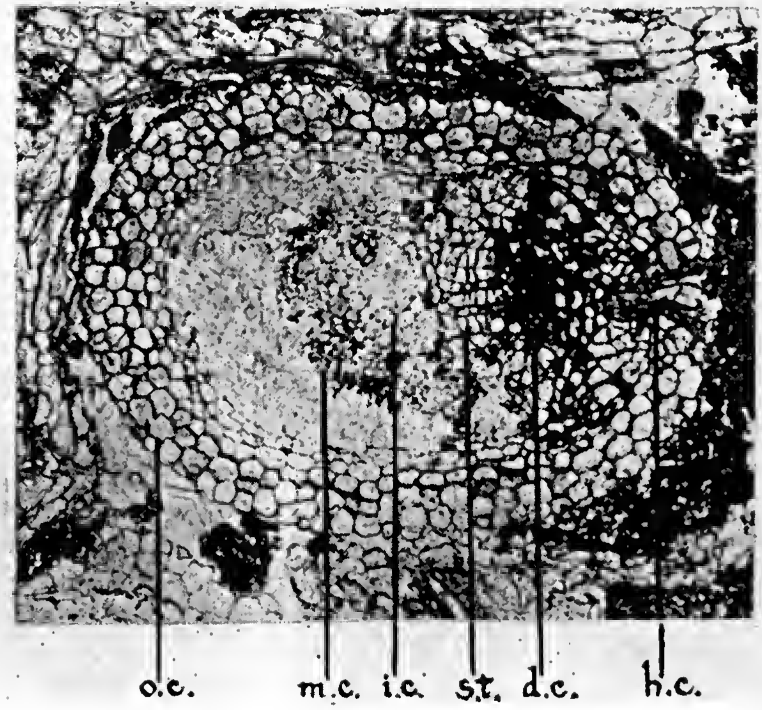
By F. E. Weiss, D.Sc.

[With Figs. 66 and 67.]

SOME time ago I had noticed in a Stigmarian rootlet the formation inthe middle cortex of a certain amount of secondary tissue (see fig. 66, *s.t.*), which was apparently developed under the stimulus of some injury to the rootlet. The nature of this injury puzzled me a good deal and I was particularly at a loss to explain the presence of a curious enlarged cell (*h.c.*) which had forced its way to the surface through the outer cortex. I had consequently put the section on one side hoping that further specimens of a similar kind might enable me to clear up this difficulty.

In the meantime a paper by F. W. Oliver has appeared in this

Journal[1] in which several parasitic fungi of Permo-Carboniferous Age are described. One of these which is found on the fronds of *Alethopteris Aquilina*, has been considered by Magnus[2] to agree so closely with the recent genus *Urophlyctis* that he has suggested for it the name of *Urophlyctites Oliverianus*. After referring to the various papers by Magnus and others on this interesting genus, I have come to the conclusion that a possible explanation of the structures observed in the Stigmarian rootlet is afforded by the supposition that it had been attacked by a fungus of the *Urophlyctis* type. Nor is this assumption hazardous now that we have Magnus' authority for the existence of a *Urophlyctites* at the Coal-Measure period.



Text-Fig. 66. General view of Stigmarian rootlet on slide No. Q 373 in the Cash Collection, Manchester Museum, Owens College. *o.c.*, outer cortex; *m.c.*, remains of the middle cortex; *i.c*, inner cortex enclosing the dark wood elements at the top and a large space in the position of the defective phloem; *s.t*, secondary tissue, of callus-like nature cutting off to the outside the dead and disorganised cells (*d.c.*); *h c.*, large hypertrophied cell penetrating the outer cortex and opening to the exterior.. From a photograph by Mr. A. Flatters.

The rootlet in question (fig. 66), from the Halifax Hard Bed, is contained in a preparation by Mr. James Binns made in 1891 and

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[1] Notes on Fossil Fungi; NEW PHYTOLOGIST, Vol. II., 1903, p. 49.

[2] P. Magnus. Ein von F. W. Oliver nachgewiesener parasitischer Pilz. Ber. der. deutsch Bot. Ges., 1903, p. 248.

now incorporated with the Cash Collection in the Manchester Museum, Owens College. Its catalogue number is Q 373. The rootlet is cut transversely and its section is almost elliptical, the greater diameter being about $1\frac{1}{2}$ *mm.* The rootlet is of normal type with a well preserved outer cortex (*o.c.*) consisting of some four or five layers of cells. The outermost layers are large and polygonal, while the inner ones are narrow and tangentially compressed. Some of the epidermal cells are slightly flattened, but otherwise uninjured and the root shows no signs of having undergone any decay previous to mineralisation. The middle-cortex was evidently composed of loosely set cells and is consequently as usual defective in preservation, the remains of this layer (*m.c.*) being collected around the central cylinder. These compressed cells have thin cell walls and dark contents, the latter being in some cases contracted to the centre of the cell space and connected by delicate threads to the cell walls. This middle-cortex would seem to have been composed of cells less stellate in character than those often met with in this portion of Stigmarian rootlets. The inner cortex has not a very distinct boundary, but is found clothing the vascular cylinder, the wood of which has slipped somewhat to one side owing to the defective phloem. The position of this latter tissue is as usual indicated by a large lacuna. A little to one side of this space is a curious mass of tissue partly of secondary formation (fig. 67 *i.s.t.*) and suggestive, if one were dealing with a recent rootlet, of an effort on the part of the plant to heal up a wound or to protect itself against further ravages by some parasitic organism. In one or two places the secondary tissue seems in contact with the inner cortex, but it seems to have taken its origin from the cells of the middle cortex. That a secondary division of cells can arise from this layer of Stigmarian rootlets I have shown[1] in another rootlet, in which, however, the divisions arose in the outermost layers of the middle cortex, while in the present case the inner layers seem to have become meristematic. The secondary tissue is fairly regular in its median portion, but becomes less regular towards the edges. The cells are for the most part narrow and thin-walled and resemble closely the cells produced in the formation of callus or wound cork in recent plants.

This secondary tissue has cut off towards the outside a large patch of dark and disorganised cells, which are considerably compressed, so that their contents are rendered almost irrecognisable.

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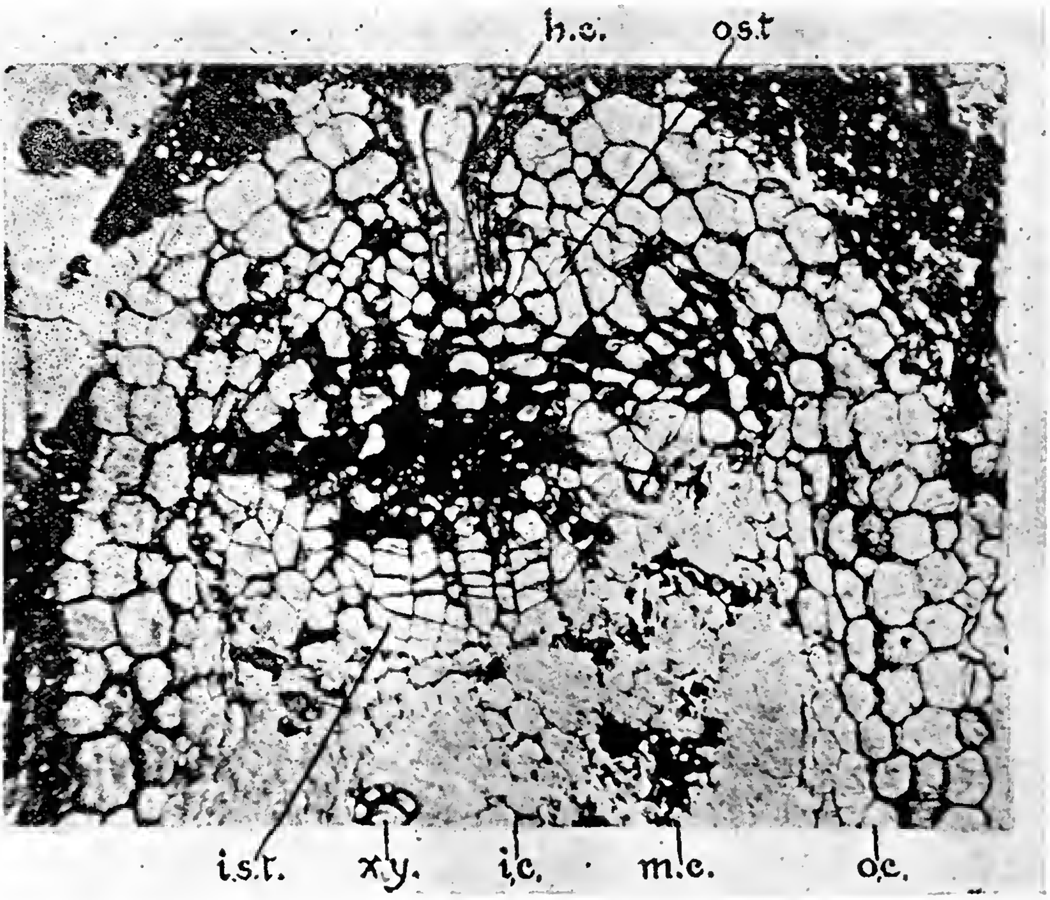
[1] F. E. Weiss. Manchester Memoirs, 1901.

Nothing can be seen in them that could be definitely identified with fungal hyphae, but if, as is suggested, the fungus was of Chytridiaceous affinities the mycelium may have been of a very slight and transitory kind and would very probably not have been preserved. At the outside of the dark patch we see another group of cells which show signs of having undergone a process of division (*o.s.t.*) These, however, are more irregular in their arrangement than the cells of the inner secondary tissue; their cells are less flattened and have thicker walls At the side, however, indicated by the lettering (*o.s.t.*, fig. 67) the secondary nature of the tissue is quite apparent. It seems to have taken its origin from the innermost layer of the outer cortex, which, as mentioned above, is of a more delicate character than the outer layers of that tissue.



Text-Fig. 67. Enlarged view of a portion of the above rootlet containing the large hypertrophied cell *h.c.* The latter is widely open to the exterior and is seen to contain a spore-body. The oblique line across it is due to a crack in the matrix and is not a cell wall.

o.s.t., outer secondary tissue caused by divisions in the inner layers of the outer cortex.

i.s.t., inner secondary tissue formed from the middle cortex, and cutting off a mass of dead and disorganised cells.

xy., xylem elements.

i.c., inner cortex, *m.c.*, middle cortex, *o.c.*, outer cortex.

Springing from this outer secondary tissue and in apparent organic connection with it is the large cylindrical cell (*h.c.*) alluded to above, which lies in a deep pit-like depression of the outer cortex. The passage through the outer cortex is considerably wider than the cylindrical cell, and is lined by the remnants of dead cells which seem to have been destroyed by the outward growth of the hypertrophied inner cell of the cortex. The latter cell was therefore probably of greater width than is now shown in the preparation, and may be supposed to have undergone considerable collapse. This view is borne out by its appearance, which, especially in the central contracted region, is suggestive of a shrunken cell. Here it is only $\frac{1}{40}$ *mm.* in diameter, while at its apex it is nearly twice as broad. The length of the cell is $\frac{1}{4}$ *mm.*, and at its apex is a wide aperture through which the bulk of the contents would seem to have escaped before mineralisation, and this would be a sufficient cause for the contraction which has evidently taken place in this cell. It seems also to have contracted slightly in length as it does not now reach to the outermost limits of the outer cortex.

The nature of the contents of this cell may, perhaps, be indicated by what are I think possibly remnants thereof. Half-way down the hypertrophied cell there is a fairly large round body not unlike a very thin-walled spore (20μ), and at the base of the cell a shrunken membrane would seem to have surrounded another spore. One or two faint indications of other spore-like bodies can be seen in other focal planes. Remains of other contents are indicated at the base of the cell by fine thread-like granulations, but their nature is too uncertain to allow of identification. The appearance of an oblique wall crossing the hypertrophied cell near its distal end is due to a crack in the matrix and does not represent any cell-membrane. As the cell is therefore open to the exterior it might, perhaps, be argued that the spore-like bodies had entered from the outside, or were due to some fungus which might have developed its spores in this convenient cavity without having preyed upon the plant. Such an explanation might be sufficient if the cell in question were the normal part of a Stigmarian rootlet. As it is, however, this hypertrophied cell itself requires explanation.

Two alternatives are suggested by the occurrence of spore-like bodies within the cell, and by the formation of secondary tissue within the root with the apparent object of limiting the ravages of some parasitic organism. The large cylindrical cell might either be a fungal sporangium or an hypertrophied cell of the rootlet acting as such. The latter view would seem the more probable both by reason of the organic connection of the cell with the rootlet and from the similarity of its walls with those of the rootlet. If this should be the correct explanation, a fungus of the *Urophlyctis* type at once suggests itself as the probable cause both of the hypertrophied cell and also of the development of a callus-like tissue.

For most plants attacked by *Urophlyctis* are stimulated to produce gall-like swellings in the formation of which active secondary growth of a similar kind to that seen in the Stigmarian root takes place. Here, however, the proliferation is internal probably owing to the almost hollow condition of the rootlet, just as is often the case in the Dandelion when attacked by *Protomyces*.

Magnus has given us a very good account[1] of the parasitic fungi which attack the underground organs of plants and of all those enumerated and described by him the genus *Urophlyctis* seems to cause disturbances most like those seen in our rootlet. There are of course differences between the two. In the specimens described and figured by Magnus in the Annals of Botany[2] for example it will be seen that the hypertrophied cell, which contains the resting spores in the genus *Urophlyctis*, seems organically connected with the host plant throughout its whole extent, and does not therefore contract when its contents have escaped, but retains its oval shape. This and other differences might, however, be expected of a fungus which, however closely allied it may be to *Urophlyctis*, is so widely removed from it in point of time.

The genus *Urophlyctis* is a parasitic fungus attacking the leaves and roots more particularly of marshy plants or of plants inhabiting regions which are flooded from time to time, and it would therefore seem quite reasonable to assume that the corresponding Palæozoic genus *Urophlyctites*, represented so far by the species *Oliverianus*, which infests the leaves of *Alethopteris*, might also be found to attack Stigmarian rootlets which we believe to have grown under conditions that must have been peculiarly favourable for this kind of fungus. If, as I think most probable, this was the case, and if the interpretation given above of the features observed in this Stigmarian rootlet should prove correct, I would suggest the name of *Urophlyctites Stigmariae*, for this problematical fungus.

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[1] Abhandlungen des Bot. Vereins der Provinz Brandenburg, xliv., 1902.

[2] Annals of Botany, Vol. xi., 1897, p. 87.

Owens College, Manchester. *January 28th, 1904.*

NOTE ON A SPECIES OF *CHARA* WITH A SPINOUS CORTEX.

By Arthur W. Hill, M.A.

[Text Figs. 68—73.]

THE development of the cortical covering of the internodal cells of *Chara* by means of up-growths from the lower node meeting cells which grow downwards from the nodal tissue above has been thoroughly described by Sachs and Migula. A reference to the figures of *Chara fragilis* in Sachs' Text-Book or to the figures of cortex development in Rabenhorst's Kryptogamen-Flora will obviate therefore any detailed description.

The cortex of the specimen, which forms the subject of this note, agrees generally with that of *Chara fragilis* in that the "middle rows" of nodal and internodal cells are separated from

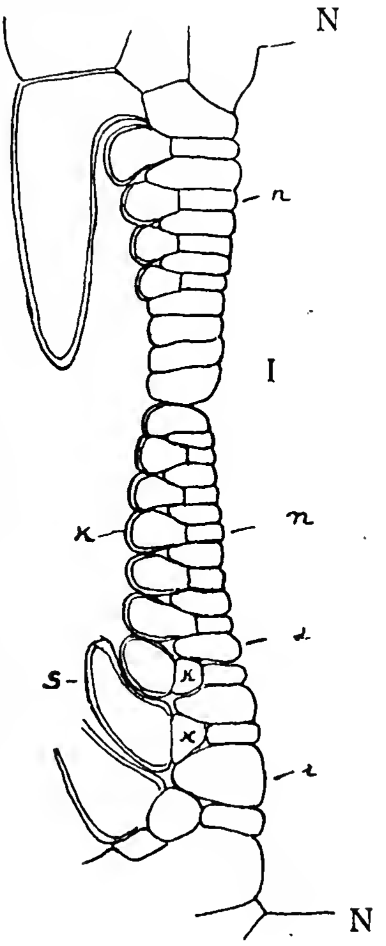


Fig. 68.

Median longitudinal section of a young internode (I), with the cortical lobes developed from the nodes (N) already divided up into nodal (n) and internodal cells (i). The latter have produced head cells (K) by division and on these the spines (S) are borne.

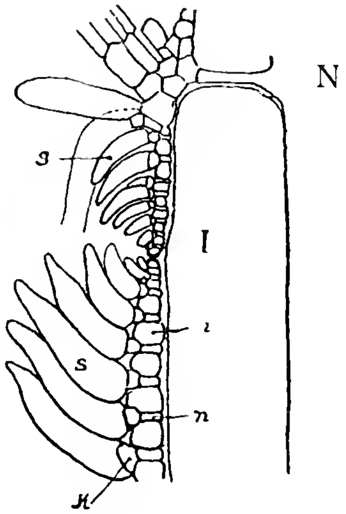


Fig. 69.

An older stage than Fig. 68; as before, the cortical covering of one side only of the internodal cell (I) is figured.

one another by two rows of plain cells (cf. Rabenhorst, Bd. V., Fig. 16).

The chief interest lies in the spines, which are developed from the nodal cells of the "middle rows" of the cortex lobes.

A median longitudinal section of a young internode (Fig. 68) shews the ascending and descending lobes to be already divided up into nodal (*n*) and internodal cells (*i*), the nodal cells having cut off by a tangential division a head cell (*k*) from which in some cases a spine (*s*) has already been produced.

Owing to the ascending lobe being older than the descending one, it is in all cases found to be in the more advanced stage of development, as a comparison of the spines from the two lobes will shew.

The spines of both ascending and descending lobes are developed in regular acropetal succession, and are impressed with the direction of growth of their respective parental lobes.

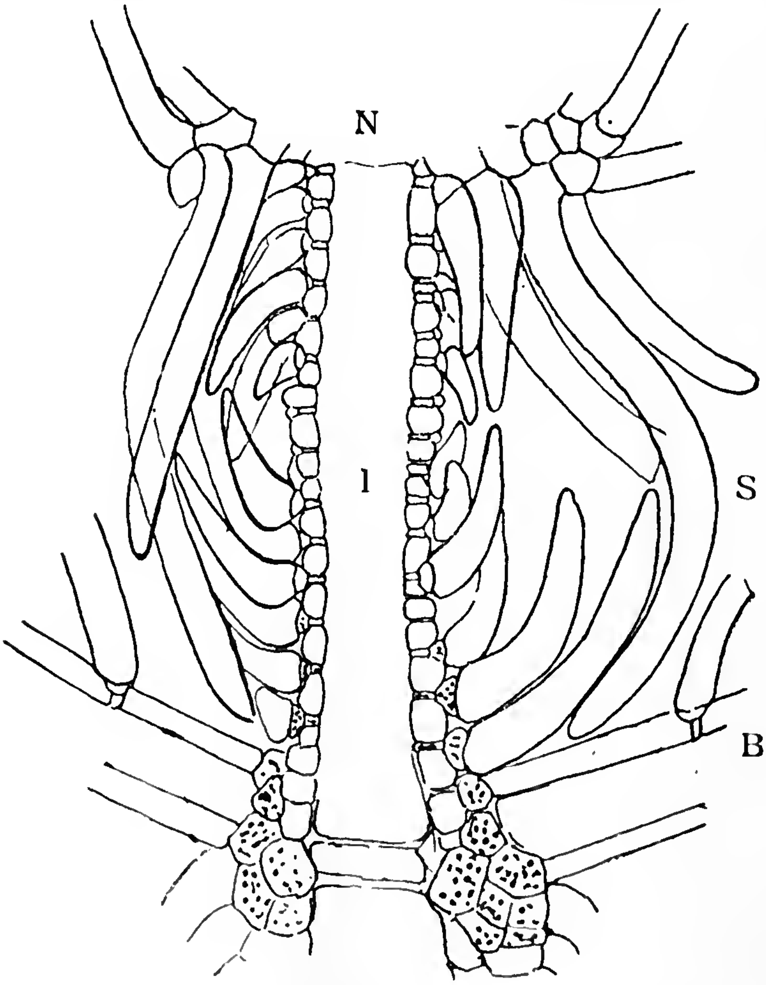


Fig. 70.

An internodal cell about the seventh from the growing point, shewing branches [B] given off from the nodal complex; the distribution of starch is indicated by dots.

The largest and oldest spines are in each case situated nearest to the nodes of the stem, whilst they are quite small where the two lobes come in contact, usually at a point about two-thirds of the way up the internodal cell. And since the spines from the ascending lobe bend sharply upwards, and those from the descending lobe bend downwards in an equally striking manner, the tips of the spines in the young state tend to meet one another and give the section of a young internode a peculiar and very characteristic appearance (Fig. 69).

An examination of an older internode (Fig. 70) shews the spines in a further state of development and considerably elongated, so that they have over-lapped each other and become interlaced, thus forming a tangled barrel-like figure of spines, which makes a dense protective covering to the young internodal cells.

With the increasing age and consequent lengthening of the main internodal cells, the spine-bearing nodal cells of the cortex become separated from one another and the spines which are borne in groups of three tend to lose their characteristic arrangement.

The Figs. 71, 72, and 73, shew old nodal cells of the cortex in longitudinal and transverse section and also in surface view; they are slightly different to the figures previously published in Rabenhorst.

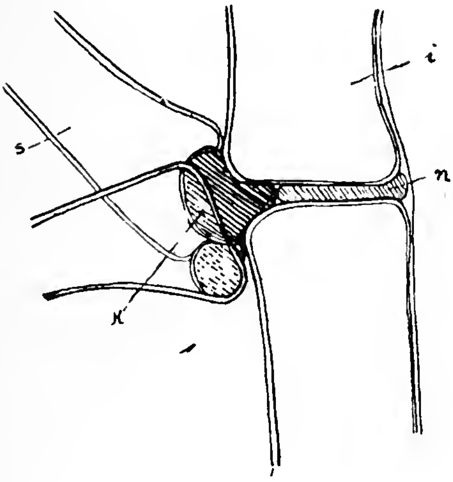


Fig 71.

Fig. 71.—Longitudinal section of a fully developed cortical node with spines. The starch in the nodal cell (*n*) and head-cell (*k*) indicated by shading.

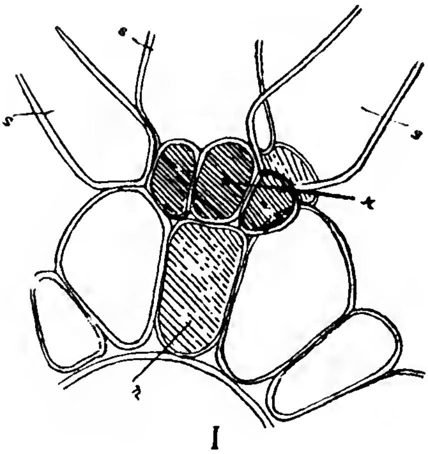


Fig. 72.

Fig. 72.—Transverse section of the cortex shewing a nodal cell (*n*) with head-cells (*k*) and group of spines. Shading indicates occurence of starch.

The small nodal cell (*n*) has produced by a tangential division a swollen head cell (*k*), on either side of which two or sometimes three lateral cells have arisen, and it is on these cushions of five or more cells that the groups of three spines are produced.

In the figure of the surface view (Fig. 73) the spines have been omitted and the other cells are represented in perspective, as seen when looking down on the surface of the cortex.

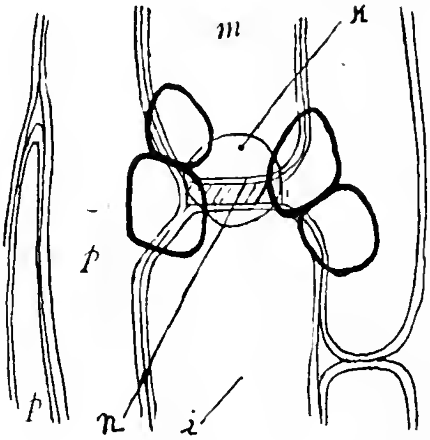


Fig. 73.

Fig. 73 —Surface view of the cortex shewing the "middle row" (*m*) of nodal and internodal cells with plain cells (*p*) on either side. The nodal group is similar to those seen in Figs. 71 and 72 in section. Spines are omitted. The nodal cell only is shaded.

The arrangement of the cortical lobes and spines in *Chara* seem to me to be especially interesting from the physiological point of view with reference to recent theories of geotropism, since those which cover any given internodal cell behave in a diametrically opposite manner, according as they have arisen from the upper or lower nodal complex.

An examination of the starch contained in the tissues by means of iodine solution has shewn that its distribution is quite striking and peculiar.

I have been unable to demonstrate starch in the youngest portions of the stem corresponding to the stages represented in Figs. 68 and 69, or in even more fully developed internodes. In older portions of the stem starch grains can be clearly seen, but their distribution is found to be curiously localized.

Large and conspicuous starch grains are found only in the cells forming the nodal complex of either stem or branch or in the cells forming or developed from the nodal cells of the cortex (cf., Figs. 70, 71, and 72 where the starch-bearing cells have been shaded—the starch is not indicated in the nodal group of Fig. 73).

Starch grains do occur in quantity in the chlorophyll granules of the large internodal cells of the main stems, but they are small and inconspicuous and are quite different in appearance from those of the nodes. Minute starch particles may also be found in the chlorophyll-corpuscles of the older internodal cells of the cortex and cortical spines.

No other bodies, which might function as statoliths, like the refractive bodies recently described by Giesenhagen[1] and Schröder[2] in the root-hairs of certain species of *Chara*, have been detected in the lobes or spines of the cortex of this species. This agrees with Schröder's results, since he also failed to find these bodies in the stem-cells.

Although the conspicuous starch grains are developed in some abundance only in the nodal cells in the region of which the cortical lobes are developed, and also in the cortical nodes from which the spines are developed, it does not appear that this peculiar distribution of the starch has any relation to the characteristic phenomena of growth exhibited by the cortical lobes and spines.

It seems probable, therefore, that normal geotropic stimuli have little or no influence on the direction of growth of the cortical lobes and spines, whether of the ascending or of the descending order; but that each internodal cell, and its concomitant nodal portions, together with the cortex and spines, forms a separate and definite system on a minute scale, complete in itself, with its own centre of attraction,—marked by the zone where the cortical lobes meet together,—towards which its members grow in opposite directions with conspicuous regularity.

[1] Giesenhagen. Ber. d. Deutsch Bot. Ges., 1901. Bd. xix., p. 277.

[2] Schröder. Beihefte z, Bot. Centr., 1904. Bd. xvi., p. 278.

King's College, Cambridge. *Feb. 27th, 1904.*

A FOUNTAIN ALGA.

By L. Beesley, B.A.
(Dulwich College).

[Plate 1 and Text Figs. 74 and 75.]

In certain of the pools in the neighbourhood of Fleam Dyke, at Fulbourne, in Cambridgeshire, subterranean springs make themselves evident as small fountains, which keep the particles of sand and chalk in constant movement.

During one of the Botanical Excursions in the Long Vacation of 1902, my attention was directed by Professor H. Marshall Ward to the peculiar green colour of many of these submersed fountains, due to an Alga which is constantly associated with the fountains—and the fountains only—in a particular pond, and it was suggested that I should attempt the cultivation and determination of the Alga, since it appears to have been hitherto undescribed.

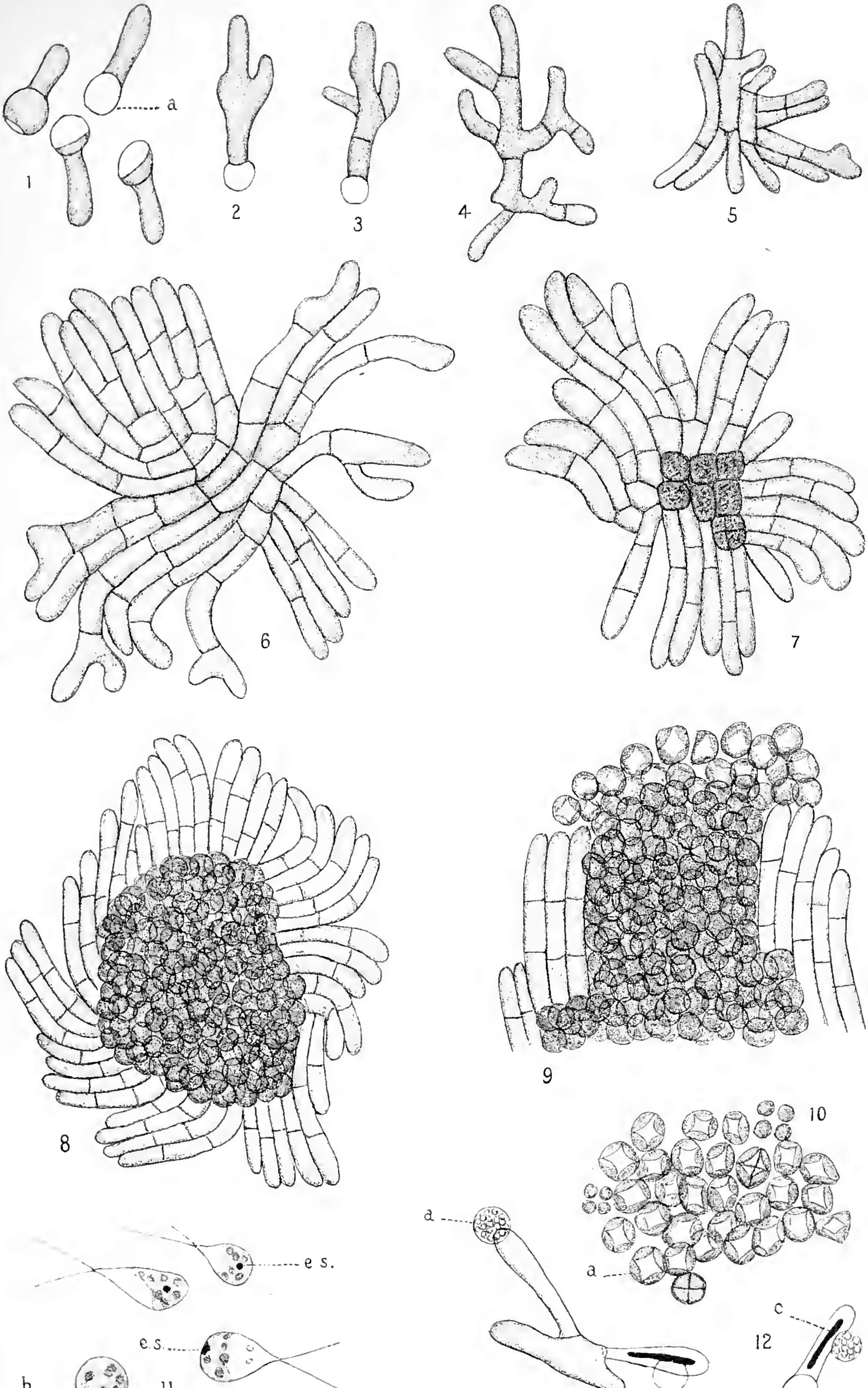
The particular habitat of the Alga referred to above is a pond about 12 ft. in diameter with a general depth of 15 ins. From this pond a small stream flows to join a large dyke, which dyke drains the surrounding fields.

Through the floor of the pond numerous small springs bubble up continuously, like fountains, and drive the particles of sand, etc., into active motion, and in the throat and neighbourhood of these fountains small green particles are seen which are constantly kept moving by the issuing current of water. In places the whole of the floor is covered with these green particles and being kept always in motion by the bubbling spring presents a most interesting and beautiful appearance.

The general temperature of the water in the pond was 11·4°C, while the temperature of the dyke into which it emptied was found to be 13·5°C.

The green particles on examination were found to consist almost entirely of grains of sand and flint—of about the same size as ordinary sea-sand—on the surface of which the thallus of the Alga spreads itself as a covering.

There are other springs to be found near these, but in none of them does the particular Alga appear to grow. In one of them, however, there are similar grains of sand on which many different species of Diatoms grow in great profusion and attain a very large size.



Highley lith & Imp. L.B. & R.W del

BEESLEY — A FOUNTAIN ALGA.

In July the surface of the pond was clear of plants and weeds and light could easily penetrate to the bottom, but later in the year a thick felted mass of watercress covered the surface, in sufficient quantity apparently to prevent any light reaching the bottom. No difference could, however, be detected in the macroscopic or microscopic appearance of the Alga under the changed conditions.

The first examination of the grains showed that a particular species of Alga, coating the grains, is concerned in all cases. Owing, however, to the impossibility of studying the detailed structure of the Alga on the irregular surface of sand grains, it was suggested that an attempt should be made to imitate the natural conditions as nearly as possible by means of artificial springs in the laboratory.

The following apparatus was fitted up in the laboratory, and by its means the Alga was induced to grow. A large bell-jar was inverted and to the narrow neck was fitted a cork, through which passed a piece of glass tubing drawn out to a small hole. This tubing was connected to a tap and a miniature fountain was thus produced. Quantities of sand covered with the Alga were placed in this fountain and were kept in motion by the current of water, thus imitating to a certain extent the natural conditions. It having been shown that the Alga will grow under these conditions, glass coverslips were placed vertically in the the throat of the fountain, with one end embedded in the sand grains, the argument being that if the Alga will grow on silica it will grow on glass.

The Alga grew readily on coverslips, and these, when required, could be removed and placed on a culture cell, where the life-history of the Alga could be observed.

The above apparatus was in use for about three months, but was found inconvenient for several reasons—the chief one being that while the sand grains in the centre were exposed to the current of water, those at the sides of the bell-jar were at rest and not bathed by fresh quantities of water, and thus the natural conditions were not completely imitated.

A second and improved form of apparatus was then fitted up (Fig. 74). This consisted of a glazed porcelain trough, on the bottom of which was placed a flat spiral of lead composition tubing of $\frac{1}{4}$ in. internal diameter. This spiral was pierced at intervals of about one inch with fine holes on the upper side. Both ends of the spiral were open and connected by rubber tubing with a tap. The trough was

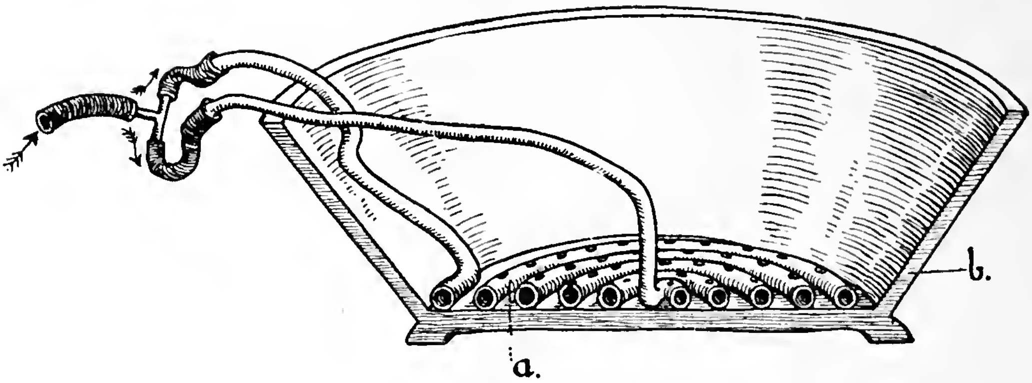


Fig. 74.

Diagram of Second Form of Apparatus, in median longitudinal section. *a*, lead spiral; *b*, porcelain trough.

half filled with Alga-covered sand grains, and the tubing thus covered by a layer of about 6 ins. of sand. When a current of water was turned on, about twenty small springs were formed, and the whole mass of sand grains was kept bathed and in motion by fresh supplies of water, thus more nearly imitating the natural conditions. Coverslips were placed upright in the sand, on which the Alga grew freely.

For the study of the life-history of the Alga on the coverslips the following method was employed:—A culture cell (Fig. 75) with rather wide arms was selected and firmly cemented down to a glass slide by means of sealing-wax. Through one of the side arms of the cell was passed a piece of fine glass tubing, which was turned

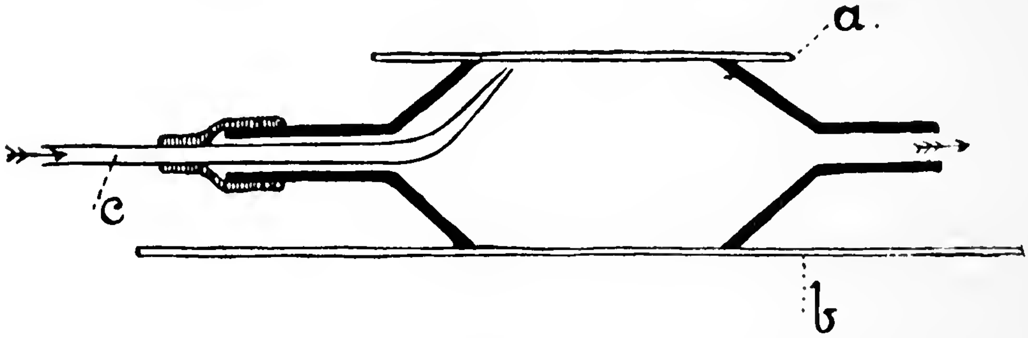


Fig. 75.

Diagram of culture-cell and method of culture. *a*. Coverslip with Alga growing on the under surface; *b*, glass slide; *c*, fine glass tube through which water is supplied to *a*.

upwards at the end as it entered the cell and ended in a fine hole, so that this hole was just on a level with the upper open surface of the cell. A rubber joint made connection between the glass tube and the arm of the cell. Coverslips with the Alga growing on them were placed face downwards on the open mouth of the cell so that the turned-up end of the glass tube was just touching the coverslip. A current of water, controlled by a screw-clip, was passed through the fine glass tube and emerging by the small hole spread out all over the coverslip, thus bathing equally all parts of it. The suction produced inside the cell kept the coverslip firmly in position, and obviated the employment of any vaseline. By this means the growth of the Alga could be followed from day to day and its life-history made out, without the coverslip being removed.

This life history is most easily traced from the zoospores. which were discovered to be readily formed under the conditions above described.

The germinating zoospore (Plate 1, Fig. 1) gives rise to a short filament, which by lateral budding grows into an irregularly branched thallus of a star-shaped or circular form with filamentous branches joined laterally to one another to form a compact disc, all radiating out from a common centre (Figs. 5, 6, 7).

The thallus is flattened and closely appressed to the substratum, to which it is firmly fixed, and even a very strong fine stream of water directed straight on the thallus fails to dislodge it or cause any displacement.

It is at first but one cell thick, and although it may be subsequently thicker, it never forms erect outgrowths or branches.

The first division wall of the germinating zoospore is at right angles to the long axis of the young filament, but lateral buds may arise before any division walls are seen (Fig. 2). The empty case from which the filament grew may be seen for about seven or eight days after germination, but eventually disappears (Figs. 1, 2, 3).

The individual cells are longer than broad, and the long axis is coincident with the axis of the filament.

The tip of a filament is rounded, and not swollen or pointed.

The chloroplast is a parietal plate extending all round the internal wall of the cell and is of a bright green colour. There are no visible pyrenoids.

In about a month's time the germinating zoospore has grown into a more or less star-shaped thallus (Fig. 6) at present but one cell thick, the filaments of which are composed of cells much longer than broad.

About this time one or two cells in the centre of the thallus may be seen to be differentiated from the other cells (Fig. 7). Their walls become thicker, darker and more easily seen, and the chloroplasts, hitherto parietal, become fragmentary and scattered all over the cell. The colour of these cells also becomes much darker, forming a striking contrast to the lighter green of the filaments. The cells themselves are of equal diameter all ways, of a roughly oval or spherical shape, and show a distinct tendency to round themselves off from one another.

This rounding off of the cells always begins almost exactly in the centre and proceeds outwards along radial lines, gradually breaking up the filaments into the rounded cells of darker colour. This breaking up, however, does not as yet reach to the end of the filaments.

By this time the cells in the centre which first became rounded off have divided by transverse walls at right angles into four cells. These at first grow into about the same size as the cell from which they were formed and then divide again into four. This division goes on continuously in all the cells which have become rounded off, so that at this period the thallus, at first one cell thick, now presents the appearance of a piled-up mass of dark-green cells in the centre several layers thick, surrounded everywhere by filaments still undifferentiated and only one cell thick. The colour contrast between the thickened central mass, which under a low power appears almost black, and the encircling lighter green filaments is very striking (Fig. 8).

About this time—*i.e.*, seven to eight weeks from the germination of the zoospore—zoospores may be seen to issue from some of the central cells of the thallus. Apparently these zoospores are never produced from the encircling filaments.

The breaking up of the filaments now begins to spread nearer and nearer to the edge of the thallus, until finally at one point two or three of the encircling filaments are broken up right to the tip into the rounded cells and a "break" is formed in the hitherto continuous margin of lighter green undifferentiated filaments.

It seems probable that there must exist considerable tension in the central mass of cells, for at the point where the break is formed the central rounded cells "flow out" laterally quite a considerable distance from the margin of the thallus (Fig. 9).

These rounded cells are now seen to present a marked palmelloid appearance, being quite separated and distinct from one another, and of a spherical or ovo-spherical shape. The chloroplasts are typically four—and may be five or six—parietal, convex, with the convex edge turned inwards (Figs. 9, 10).

These palmelloid cells may at once divide into four zoospores or into four daughter cells which may grow and again divide (Fig. 10).

The escape of zoospores is best observed in the palmelloid stage where the cells are not piled on one another. A single cell when watched in the act of forming zoospores, appears to lose the distinctness of its walls, until when the first sign of movement of the contained zoospores is observed, the walls seem to have disappeared. The contents divide into four, as said, and the ball of four zoospores begins to move slowly, turning over, and the zoospores may be seen writhing over one another. Their movements become more violent, and they begin to jerk rapidly about, and although nothing can be seen which prevents their becoming free, it is evident from their movements that they are still held fast in some way—probably by their cilia being imbedded in some mucus formed from the broken down cell. They finally become free, although one may be held fast for a considerable time after the others have departed. No remains of an envelope from which they have escaped can be seen. The time taken to become free from the first sign of movement is about five minutes.

The zoospores (Fig. 11) are pear-shaped with two cilia at the anterior narrow end. The anterior end is colourless and clear, except for two colourless globules sometimes seen, which may be vacuoles. The posterior end contains the chloroplast, which is basin-shaped, and under a high power appears to consist of numerous oval chlorophyll granules. There is a well-marked red eye-spot in the posterior end.

The zoospores differ slightly in size, but all appear to have the same morphological value—*i.e.*, they all germinate into a new thallus without any conjugation.

After swimming about vigorously for a short time occasionally being arrested through the cilia becoming entangled with diatoms, etc., the zoospore comes to rest, attaching itself by its front end to the coverslip, a diatom, or the edge of a thallus. The colourless anterior end bearing the cilia is immediately drawn up, so that the zoospore assumes a circular form and appears uniformly green—*i.e.*, without any colourless portion (Fig. 11b.)

It commences to germinate immediately and in twenty-four hours has pushed out a short tube, which elongates rapidly, so that in forty-eight hours all the contents of the zoospore have passed into the germinating tube and the circular wall of the empty zoospore may be seen very clearly (Fig 1a). The green contents may be seen passing slowly out from the zoospore into the tube: This tube is the first filament and by lateral budding grows into the the thallus, from which again zoospores are formed.

The life-cycle from zoospore to zoospore was watched day by day for many generations of zoospores, and no other method of reproduction was observed. Zoospores continue to be formed right through the winter, and no resting zoospores have been observed. They are not, however, formed in such large numbers during the winter, and do not germinate so readily.

Many different species of diatoms grow on the coverslips along with the Alga, and filaments of the thallus in their growth may penetrate beneath the diatoms, or if the latter are small, may grow completely round them and enclose them. Many of these diatoms are of relatively very large size and may grow in enormous numbers, and it may be noted in passing that in January and February, 1903, they lost all their brown colouring matter and the contents appeared a vivid green. This was common apparently in most of the different species, and was of such frequent occurrence that coverslips were covered bright green to the naked eye solely by the diatoms.

The copious growth of diatoms – which require a large amount of silica for their composition—the firmness with which the Alga becomes attached to glass coverslips, the fact that in the natural conditions the alga grows on grains of sand and flint, all point to the conclusion that the Alga is favoured by silica. This is confirmed by the fact that while the Alga grows on glass coverslips immersed in the trough, it will not grow on the inner porcelain surface of the trough itself.

It is interesting to notice that this Alga is attacked by what in all probability is one of the Chytridiaceous fungi, although the position of this fungus has not yet been determined.

There may arise either from the tip of a filament of the Alga, or laterally, a small circular swelling or "blister," which grows slightly in size until it is about as large as a zoospore of the Alga. It is quite colourless, and its contents are twelve to twenty minute colourless spores. In time this sporangium bursts and the spores issue, the containing envelope with a rent in the wall being quite visible. In running water, these spores are at once swept away, and it seems reasonable to suppose that they are carried by the water current to fresh filaments of the Alga, and so infect them. This fungus may be seen attacking the Alga in all stages, from the fully grown thallus to the young filament which has just issued from the germinating zoospore (Fig. 12). The contents of a cell so attacked turn reddish-brown, plasmolize to the centre and the cell dies. The life history of this fungus is at present under more detailed observation.

In the general shape of the thallus, and the form of the chloroplast as a parietal plate, this Alga bears a striking resemblance to the various species of *Gongrosira*,[1] but unlike them, it is not in any way encrusted with carbonate of lime—on the contrary its natural substratum is a siliceous one. Also the zoospores are not formed in terminal and swollen zoosporangia in large numbers as in the Gongrosireae. Still on the whole it appears to agree more with the Gongrosireae than with any other group.

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[1] Chodat—"Les Algues Suisses."

SUMMARY.

1. A new fresh water Alga.

2. Siliceous in habit.

3. Grows in running water of low temperature.

4. Forms an irregularly-branched star-shaped or circular thallus, the cells of which are longer than broad, containing a parietal chloroplast and no pyrenoids.

5. By continuous division, beginning always at the centre of the thallus, the cells break up into zoosporangia, from which four zoospores issue.

6. The thallus may pass into a palmelloid condition, and in this condition the cells divide at once to form (*a*) zoospores, or (*b*) daughter cells which repeat the process.

7. Bi-ciliate, pear-shaped zoospoores, with basin-shaped chloroplast, red eye-spot and colourless anterior portion.

8. Zoospores germinate without any period of rest into a new plant.

9. No sexual stage known.

The work on the above Alga was carried out in the Botanical Laboratory at Cambridge from July, 1902, to Christmas, 1903, and my sincere thanks are due to Professor H. Marshall Ward for permission to work in his laboratory and for his kind advice and encouragement. I have also to thank Mr. R. Wood, of Gonville and Caius College, Cambridge, for kind help with the drawings and diagrams.

EXPLANATION OF THE FIGURES ON PLATE I., ILLUSTRATING MR. BEESLEY'S PAPER ON "A FOUNTAIN ALGA."

Fig. 1. Zoospores germinating. *a*—Empty zoospore case. × 950 (about).

Fig. 2. Young filament with lateral buds forming. × 625 (about).

Fig. 3. Transverse walls formed. } × 625 (about).

Fig. 4. Further stage of Fig 3. }

Fig. 5. The lateral budding is beginning to result in the filaments being appressed to one another, and so tending to form a flat plate. × 500

Fig. 6. Fully-grown thallus, just before the appearance of the internal division in the thallus. × 950 (about),

Fig. 7. The cells in the centre of the thallus are rounding off and one may be seen dividing into four. × 950 (about).

Fig. 8. A thallus, consisting of a piled-up central mass of rounded cells surrounded by filaments of lighter colour. × 625 (about).

Fig. 9. Portion of edge of a thallus, showing internal rounded cells issuing out from the interior of the thallus. × 950 (about).

Fig. 10. Group of cells in the palmelloid condition—some are dividing into four. *a*—Convex chloropalst. × 950 (about).

Fig. 11. Zoospores. *b*—A zoospore come to rest and rounded off. × 1100 (about).

Fig. 12. Alga attacked by a fungus. *a*-Sporangium. *b*—Spores of the fungus. *c*—Dead contents of Algal cell. × 950 (about)

THE BOTANICAL INSTITUTE AT MUNICH.

BY AN ENGLISH RESEARCH STUDENT.

IN Munich all the various appurtenances of "pure botany" are concentrated in one Institute, which stands with the adjacent palm houses in a corner of the small Botanic Gardens in the centre of the town. This is exceedingly convenient, for all the resources of the University are at once at hand, and no time is wasted in going to and fro' between library, museum, garden and laboratory.

One would not, in fact, go far wrong in taking the Munich Institute as a model of what a Botanical Institute should be. Professor Goebel of course considers that there is a general want of space, but that is because he is the Professor of the Department; to an outsider, and particularly to one used to the close quarters of a London laboratory, there seems to be a palatial spaciousness about the buildings. These were rebuilt and fitted according to Professer Goebel's directions, and in the number of practical conveniences one sees the advantage of having a master mind to settle even the trifling arrangements of such a building.

On the ground floor is the lecture room, holding about 300, while beyond it are the museum and herbarium, as well as various professors' and assistants' rooms. Along the corridor is a door leading directly into the big palm house, which is exceedingly convenient for students who are working with living plants.

On the next floor are the Professor's suite of rooms and small Conservatory, the Library, Professor Giesenhagen's room, and the Arbeit Saal or Research Laboratory, which is large and well lighted. Here the arrangements are quite simple but very efficient; each student has a table with electric lamp, gas, and such reagent bottles and material as he may require, with a cupboard on the wall beside him. In the middle of the room are two large tables used for the microtomes, and along the walls without windows are—a work table for paraffin embedding, paraffin ovens, two water taps, a table with large reserve bottles of absolute alcohol, etc., a fume cupboard for chemical work and a cupboard of re-agents. Opening out of the big laboratory is a dark room, which, with the camera are used by the students. The beautiful brown parquet floors afford me much delight, and form a striking contrast to the bare ink-stained boards common in England. The still larger general laboratory and attendants' room are on the second floor, and are very similar to those below.

In the surrounding gardens, which are unfortunately quite small, are several greenhouses with most of the plants usually to be found in Botanic Gardens. One house is particularly striking, being is entirely devoted to a grove of tree ferns, arranged in as natural a manner as possible; these were brought back by Professor Goebel from his travels and are a particularly charming and unusual addition to the houses.

The Professor lectures every day on *Allgemeine Botanik*; these are the only lectures he gives this term and are attended by about 300 students. The lectures are perforce somewhat elementary, but they are interestingly and broadly treated, and are splendidly illustrated with a wealth of experiments, diagrams, lantern slides, dried and spirit specimens, and numerous plants from the houses. The immense advantage of having the contents of museums and houses available for the lectures is borne in on one.

In the "Arbeit Saal" the students engaged in research for the Doctorate in Botany, and foreign research students' work. Here the true greatness of the Professor comes out best. He encourages each to work out his own ideas, for he prefers a laboratory with a variety of interest, and does not approve of a Professor forming a "school" of followers out of his students. Once or twice a day he comes round to each, frequently with the time-honoured question, "Haben sie etwas gefunden?" He goes from one subject to another in a few minutes, and enters into the point of view of each in a wonderful way, from the spermatozoids of Ginkgo (which come to the Laboratory with Professor Fujii, who is here at present) to Orchidaceæ, from Vorläuferspitze to Fern-antheridia, from Cycads to Liverworts, he gives valuable suggestions and references to papers old and new on every subject.

Professor Giesenhagen and the assistants also come round ready with help for all that ask it. In fact the student gets the most excellent treatment—he is left alone if he prefers it, but can with a word have all the resources of the laboratory at his command.

Once a week the professors, assistants, and senior students of the various departments of "applied" botany unite with the "pure" botanists to discuss recent literature, and every other week or so one of the assistants or students gives a "Vortrag" on some special subject, generally in connection with his own work. These evenings are exceedingly valuable, for they form an easy and pleasant way of keeping in touch with recent literature, and the students learn much by preparing their "Vortrag."

Amid all this excellence I see only one serious flaw, and as that is not peculiar to this particular Institute, but is due to the entire system of University teaching in Germany, it is hardly fair to speak of it. Still I do not think that the students get a sufficiently thorough general and practical training before they are forced to become specialists by preparing their degree "Arbeit"; nor are advanced lectures utilised to the extent they might be as a means of teaching. In my training the advanced lectures afforded me such delight that I deeply sympathise with the unsuspecting German students who are foregoing a similar pleasure.

The kindness and courtesy of the Professor and his Assistants are very great, and will not be forgotten by foreigners who have had the privilege of studying here. One can always learn something from everyone, but in this Institute, where there is such a broad-minded Professor and such an international studentship one has special opportunities. M.C.S.

München, Königl. Pflanzenphysiologisches Institut.
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STUDIES ON CYANOPHYCEAE.

BY F. E. FRITSCH, B.SC., PH. D., F.L.S.

[WITH TEXT-FIG. 76].

INTRODUCTORY.

PROBABLY no other group of the vegetable kingdom has received so little attention from morphologists, as that of the Cyanophyceae; an enumeration of the important papers published would not fill much more than a page of this journal. Much attention has been given to the investigation of the movements of the Oscillarieae and at one time or another to the cytological characters of the Cyanophyceous cell and to the structure of the sheath, but for the rest the greater part of the work has been purely descriptive of the fully-developed vegetative structure. The lack of our knowledge regarding many essential points in this group, was so obvious, and I was confronted by so many problems of interest during my examination of the Algal flora of the Royal Botanic Gardens at Kew, that I commenced a study of certain features of the group nearly two years ago. I propose to embody the results of this investigation in the present series. My material was derived from the main tank in the *Victoria Regia* house at Kew and was originally intended for the study of an *Oedogonium*, which was present in considerable quantity, blue-green Algae being only represented in negligible quantities at the time. A month or two later the *Oedogonium* had disappeared almost entirely from the glass vessel, in which the material had been placed, and had been superseded by a rich growth of blue-green Algae.

Quite recently Dr. F. Brand (1903) of Munich has published a very interesting series of observations, entitled "Morphologisch-physiologische Betrachtungen der Cyanophyceen," which are based on a ten years' study of the group in question. Many of my observations confirmed or supplemented Dr. Brand's and I have therefore decided to publish these at once and make them the starting-points of the present series.

I.—SOME POINTS IN THE STRUCTURE OF AN ANABAENA.

IN the above-mentioned material a species of *Anabaena* occurred in all stages of development and almost at once attracted my attention by the abundant occurrence of heterocysts. The only distinction between *Anabaena* and the closely-allied genus *Nostoc* is to be found in the absence of a common gelatinous investment round the colonies of the former; Hansgirg is right in my opinion in not regarding this distinction a generic one (cf. Hansgirg '84, p. 13) and in referring *Anabaena* to *Nostoc* as a sub-genus.[1] The two genera are maintained in Engler-Prantl by Kirchner ('98, p. 72), as also by Cooke ('84, pp. 224 and 234) and Wolle ('87, pp. 278 and 285). A third genus *Sphaerozyga*, characterised by a centripetal development of the spores with reference of the heterocysts, is now almost generally regarded as a section of *Anabaena* (Hansgirg '84, p. 13, '92, p. 69; Kirchner '98, p. 74.)

The species, I am about to describe, belongs to *Anabaena* proper, *i.e.* there is no common investment, although each filament is surrounded by a fairly broad sheath of highly transparent mucilage; this is generally quite evident in young stages and (Kleinenberg's) haematoxylin stains it dark blue at all times. The ordinary vegetative cells vary very considerably in size, shape and colouration. The young moniliform filaments are composed of relatively small spherical cells, more or less flattened at each end, and frequently having a very dark green (pale slate-green) colour; these young filaments are often very much convoluted (sometimes in a spiral manner), although almost perfectly straight ones are no rarity. These latter do not generally attain so considerable a length as the convoluted ones; and the appearance, assumed by the latter is probably due to the abundant cell-division, which has taken place. All transitions from these small spherical cells to larger oblong ones are to be found and both types are not rarely to be met with in the course of the same filament (cf. fig. 1); these larger and older cells generally have the typical blue-green colouration. In the large

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[1] In his Prodromus however (Hansgirg '92, pp. 56 and 67) the two genera are maintained, although reference is made to the earlier paper in a foot-note.

majority of cases the cell-contents are distinctly granular, but under certain circumstances they may acquire a homogeneous character. The heterocysts (figs. 1, 2 and 4) are, as a rule, in the species under discussion, not much larger than the ordinary vegetative cells and in certain cases may even be below the average size. They develop from the ordinary vegetative cells and are mostly to be recognised at a very early stage. The cell-contents gradually become paler, whilst a bright, highly refractive granule appears, lying apposed to one or both of the end-walls of the cell (figs. 2, 8*b* and 9); these granules are quite evident at a time when the young heterocyst still shows an unthickened wall and a distinct blue-green colouration of its cell-contents, although, as will be shown presently, some considerable alteration has already taken place in them. Except for this terminal granule the contents of the heterocysts at this stage are homogeneous or very finely granular. One can very often observe, that in the cell adjoining the heterocyst, a similar granule lies in contact with the middle of the wall, adjacent to the heterocyst, being thus situated exactly opposite and in close proximity to the granule in the latter. This appearance is not confined to the heterocysts, for granules are often seen lying opposite one another in the same way in two adjoining vegetative cells. After the appearance of this terminal granule the cell-wall of the heterocyst becomes more or less thickened, the thickening apparently commencing in one or both of the end-walls, and the cell-contents become colourless; in all cases there appears to be a certain amount of contraction of the contents. Two kinds of heterocysts are to be distinguished: Terminal ones, which have a spherical shape, flattened on the side towards the vegetative cells, and intercalary ones, which are more or less barrel-shaped and flattened at each end.[1] The bright granule above mentioned, as lying apposed to the one (terminal, figs. 8*b* and 9) or both (intercalary, fig. 2) of the flat ends of the heterocysts is usually no longer represented in later stages; but similar granules, generally of large size, are often to be found, lying singly or several in number in the cavity of the heterocyst (fig. 9).[2] The end wall or walls generally develop two little lip-like prominences internally, one on each side of the pore, which thus becomes very distinct (figs. 1, 4 and 9). In old heterocysts a plate of thickening extends right across the pore of the heterocysts; under a high power this plate appears to have no

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[1] These terms are also employed by Hansgirg ('87, p. 123.)

[2] These granules in the cavity of the heterocyst are not very evident unless stains are employed (cf. below).

actual connection with the neighbouring cell-wall.—There is, however, another type of intercalary heterocyst, which is not rare in older threads. This has a more oblong shape and each end is drawn out into more or less of a point (cf. fig. 5 of an extreme case) with a very distinct pore, whilst the wall is uniformily thickened. Some of the terminal heterocysts occasionally also exhibit such characters. I have not been able to follow up the development of this type of heterocyst; possibly it arises by a transformation of the larger, oblong vegetative cells.

It will be found, that this account of the development of the heterocyst scarcely agrees with Brand's (1903, pp. 39 and 40). His observations were apparently chiefly made on *Nostoc commune* and on species of *Tolypothrix* and it is of course possible, that the details of the development may vary in different forms. No mention is made of the terminal granule, which I have found so very characteristic of early stages, whilst his account says that "Die innere Mündung des Porus umgibt sich *bald* mit einer ursprünglich nur leicht angedeuteten Membranverdickung." I do not quite understand whether this refers to the above-mentioned lip-like thickenings or whether it is meant to indicate a thickening, extending right across the pore. Further on we find the statement: "Die auffallendsten Prominenzen gehören der Membran überhaupt nicht an, sondern werden durch gewisse etwas stärker lichtbrechende Körner gebildet." This evidently refers to the granules, I have described (cf. Brand's figs. 4, 6, 10 and 11), but which, as far as I understand, according to Brand only appear later on in the fully-developed heterocyst.

The contents of the heterocysts in later stages generally occupy only the central portion of the lumen, being separated by a space, more or less wide, from the lateral walls (cf. figs. 3, 9); in most cases, however, they are in connection with one (fig. 3) or both (fig. 1) of the end-walls in the terminal and intercalary heterocysts respectively. Detached heterocysts alone have the contents contracted together in the middle, not being in contact with the wall at any point (fig. 4).

In the young filaments (in which there is no indication of spore-formation) the heterocysts occur singly and at considerable intervals from one another (fig. 1). The filaments are almost invariably bounded at one or more usually at each end by terminal heterocysts and intercalary ones occur in the course of the longer filaments. As the filament gets older it often happens, that the two vegetative cells on each side of an intercalary heterocyst become transformed into terminal heterocysts, whilst the later loses its connection with the rest of the thread. In this way the original filament has become split up into two smaller ones, bounded at the newly-formed extremities by the terminal heterocysts; such filaments are frequently found, lying close together with a loose intercalary heterocyst between them. But new heterocysts may arise in the course of a thread at points, where there are no previous heterocysts. Their formation then takes place in the following way: An intercellular substance of a dark green colour is excreted between two adjoining cells, which gradually develop into terminal heterocysts. This intercellular substance has a flat bi-concave shape (fig. 8*a*), narrow in the middle and broadening out laterally; when the heterocysts are fully differentiated this substance has become quite colourless (fig. 8*b*). Occasionally (apparently when the excretion is formed near one end of the fiament) a heterocyst is developed only on the distal side, the short proximal portion of the filament being left without a terminal heterocyst. Brand (loc. cit. pp. 39, 50-51) was able to study the formation of such intercellular substance in connection with the formation of pseudo-branches and heterocysts in *Tolypothrix penicillata*, Thur. var. *tenuis*, Hansg.[1] and also mentions its occurrence in connection with the development of hormogonia. In *Nostoc commune* (p. 52) finally Brand has observed such excretions appearing in connection with the disjointing of the heterocysts in old cultures.

In older filaments of the *Anabaena* under discussion heterocyst-formation is much more abundant.[2] I have frequently observed as many as four terminal heterocysts side by side at one extremity of the filament and in close apposition to one another (figs. 4 and 9). In such cases the distal heterocyst is often most fully differentiated, is largest and has the thickest wall (cf. fig. 4); the following ones are generally smaller and show the typical characters to a less extent. It appears as though the functions of the first-formed heterocyst

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[1] I can fully confirm Dr. Brand's observations on this point.

[2] As far as I am aware, it is not a common thing to find many heterocysts side by side in the Nostochineae. Bornet and Thuret ('80, p. 104 and Plate xxviii., Fig. 13) have described such a phenomenon in *Nostoc ciniflonum*, Tourn.; they also in their figure show the way in which two terminal heterocysts appear on either side of an intercalary heterocyst and thus give rise to two new filaments. Palla ('93, p. 546) states that the heterocysts are "auffallend zahlreich" in *Anabaena Azolla*.

ceased after a time and that then the adjoining vegetative cell became changed to take its place and so on. The fewer the heterocysts at the end of an old spore-producing filament the larger they generally are. When abundant spore-formation is going on, the cells next to the heterocysts are apparently often in an exhausted condition and if they divide, the results of their division are below the average size of the vegetative cells. Heterocyst-formation may also affect such cells and the resulting heterocysts are then also below the average size. As a rule when several heterocysts are formed in a row the first-formed ones tend to become disjointed. Long filaments, the centre portion of which is engaged in active spore-formation, and which are bounded by a number of heterocysts, not rarely exhibit a number of collapsed cells with faint blue-green homogeneous contents (necrides) next to the chain of heterocysts. In many cases the cells near the fully differentiated heterocysts are more or less homogeneous and contain few granules (cf. fig. 9.)

The filaments of the *Anabaena* were readily stained with an aequeous solution of Iodine. The contents of the cells took on a dark brown colour almost at once, whilst even half-an-hour's immersion in the stain had no effect on the heterocysts. Young heterocysts, which, as mentioned above, still have coloured contents like those of the remaining cells, are also totally unaffected by the Iodine. Is this due to a change in the contents or to a change in the permeability of the cell-wall or to both? However, not only the heterocysts remain uncoloured by Iodine; the above-mentioned collapsed cells, occurring frequently in the neighbourhood of the heterocysts, are also unaffected. I can find no grounds for assuming an alteration in the cell-wall to have taken place here, whilst the contents have visibly undergone a change. The action of an aequeous solution of methyl-green is almost directly the reverse. The contents of the heterocysts[1] in all stages of development take on a bright green colour, whilst the vegetative cells are far less prominently coloured; apparently chiefly the granules inside the latter take up the stain. The collapsed cells take up the stain in the same way as the contents of the heterocysts, thus again evincing a similarity to these latter. A short immersion

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[1] When methyl-green is brought in contact with the smaller heterocysts it causes a dissolution of the membrane and only the terminal thickenings and the contents remain evident; in the larger fully-differentiated heterocysts the membrane tends to contract irregularly (cf. Brand, loc. cit. p. 39). The action of dilute acids is similar.

in Eosin had practically no effect at all. A longer immersion of about a day, however, resulted in the granules inside the vegetative cells and spores taking on a deep red colour, whilst the general substance of the cell was far less deeply stained.[1] Even two or three days immersion produced no very visible effect on the older heterocysts, but in the young ones the terminal granule was stained just as deeply as those occurring in the ordinary cells. The collapsed cells were only very slightly affected by the stain. In a good many cases a few of the granules inside some of the heterocysts had also apparently taken on a very faint colour; and in some cases a deep red granule was discernible within the cell-contents.

The heterocysts of the blue-green Algae were at one time regarded[2] as subserving vegetative reproduction, and, as has been shown above, their formation is at times undoubtedly connected with the breaking up of a filament. Other authors have interpreted them as receptacles for the storage of reserve-substances,[3] whilst recently (1901, 1903), Brand has described the liberation of their contents as gonidia and their germination to form a new filament in *Nostoc commune* and *N. microscopicum*;[4] this latter author also ascribes a storage-function to them and considers that their contents may serve to replenish the exhausted adjoining cells. When the very marked differentiation, attained by the heterocyst, is taken into consideration, one can scarcely agree with Borzi[5] that "the heterocysts serve solely to interrupt and limit (the threads) at varying distances." In the *Anabaena* under discussion the threads are almost invariably limited by a heterocyst, so that these cells would appear to be a necessary termination of the filament. When the filaments break up into two parts, heterocysts must be formed to limit the free ends of the new filaments. Similarly when branching takes place in a *Tolypothrix*, a heterocyst is formed just above the point, at which the continuity of the filament is interrupted by the formation of the branch (cf. also Brand, loc. cit.

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[1] This was especially evident in cells which had almost homogeneous contents with few or no granules.

[2] cf. Borzi '78. p. 239, and Hansgirg '87, p. 124; and recently Kirchner '98, p. 71.

[3] cf. Hieronymus, '92, p. 483; Hegler, 1901, p. 305.

[4] Brand, 1901, pp. 154, 155. Although heterocysts were so very abundant in my material I have not been able to confirm Brand's observations with any certainty. Several cases were however found, in which it looked as though germination of the contents of a heterocyst had taken place, but the stages were always too far advanced to recognise the typical structure of a heterocyst.

[5] Borzi '78, p. 239.

p. 39). If we further take into consideration the characteristic differences in the structure of the heterocyst, according as it is intercalary or terminal in position, we are, I think, compelled to attribute more than a mere limiting function to these cells. It is also difficult to conceive of an explanation for the occurrence of a number of heterocysts side by side (as also obtains in many species of *Tolypothrix*),[1] if their function is solely to split up the thread into a number of vegetative regions.

I have shown, that early stages in the development of a heterocyst are characterised by the presence of a bright granule on the wall, adjacent to the vegetative cells, and that a similar granule is often to be seen on the adjoining wall of the neighbouring vegetative cell. In appearance and in their behaviour towards Eosin these granules are quite identical to the so-called cyanophycin-granules, which almost always occur in considerable numbers in the ordinary cells and spores.[2] In later stages these granules are no longer present, whilst granules, which stain very faintly with Eosin, are frequently to be seen amongst the contents of the heterocysts and more rarely a deeply-stained granule occurs. It seems to me therefore, as though these cyanophycin-granules wandered over into the young heterocyst from the neighbouring vegetative cells, which in later stages often appear collapsed or devoid of the granules. Inside the heterocyst the granular substance becomes modified in some way, so that it loses its former properties. Probably the change is of the nature of the formation of a reserve-substance, for when the richly-granular spores of this Alga are placed under unfavourable conditions, necessitating a period of rest, the granules also gradually disappear and the contents become more or less homogeneous. The changed granules are scarcely affected by Eosin, whereas they are now capable of assuming a green colour with methyl green.[3] With regard to the differences between the young heterocysts and the ordinary cells in their behaviour towards Iodine, the brown colouration of the vegetative cells is probably due to the staining of the blue colouring-matter; this has evidently already undergone some change in the young heterocysts and in the

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[1] During the winter of 1902-1903 a species of *Tolypothrix* with sometimes as many as six contiguous heterocysts was abundant in the Pen Ponds in Richmond Park. I was unable at the time to examine this species in greater detail.

[2] cf. also Palla ('93), pp. 544, 546.

[3] This need not only be due to a change in the contents, but also to a change in the permeability of the cell-membrane. Since methyl-green more or less dissolves or corrugates this latter, it is probably able to penetrate to the contents far more readily than Eosin.

collapsed cells, above mentioned, and is consequently no longer affected by the Iodine.

The cyanophycin-granules have formerly been regarded as a product of assimilation, comparable to the starch of higher plants. Zukal ('94, p, 282) however regarded them as distinctly differentiated portions of the protoplasm, although there seems little justification for this view (cf. Hieronymus '92, p. 484); both nitrogen and phosphorus have been stated to occur in them.[1] They certainly grow during the life of the cell, for those in young cells are small, those in older cells and spores much larger. The above assumption of the wandering over of the cyanophycin granules into the heterocysts requires the existence of a protoplasmic communication between the cells of the filament; and I think there can be little doubt now that such is the case. Borzi ('86, p. 74 et seq., Tab. III.) as far back as 1886 demonstrated such intercellular communications between the cells of a *Nostoc* and an *Anabaena*. Nadson ('95. Tab. V., fig. 55) shows very distinct connections between intercalary heterocysts and the adjoining cells in *Aphanizomenon* and *Tolypothrix*, Such communications are occasionally to be observed between the young heterocysts and the adjoining vegetative cell in the species under discussion (fig. 3), and I am also able to give good evidence for their existence between the ordinary cells. Figs. 6 and 7 show two cases, in which the contents of adjacent spores[2] are being liberated in opposite directions and a narrow protoplasmic thread connects the two (already widely separated) protoplasts. From these and Borzi's figures it is evident that these intercellular communications are far wider that those known in higher plants, although an analogy may be found amongst the Thallophytes in the Florideae. The width of the communication is such that it would admit of the passage of a cyanophycin-granule as an entirety.

I would therefore regard the heterocyst as a recipient of reserve-substances (cf. Hieronymus '92, Hegler 1901),—as an organ for storing up the contents of neighbouring vegetative cells, when these, owing to unfavourable external conditions or to active spore-formation in the central portion of a filament, become exhausted. This quite concords with Hieronymus ('92, p. 483), when he says: "Vielleicht haben die Grenzzellen ueberhaupt die Function, übermassig in den vegetativen oder Dauerzellen gebildetes Kyanophycin aufzunehmen und als Speicher für dasselbe zu dienen."

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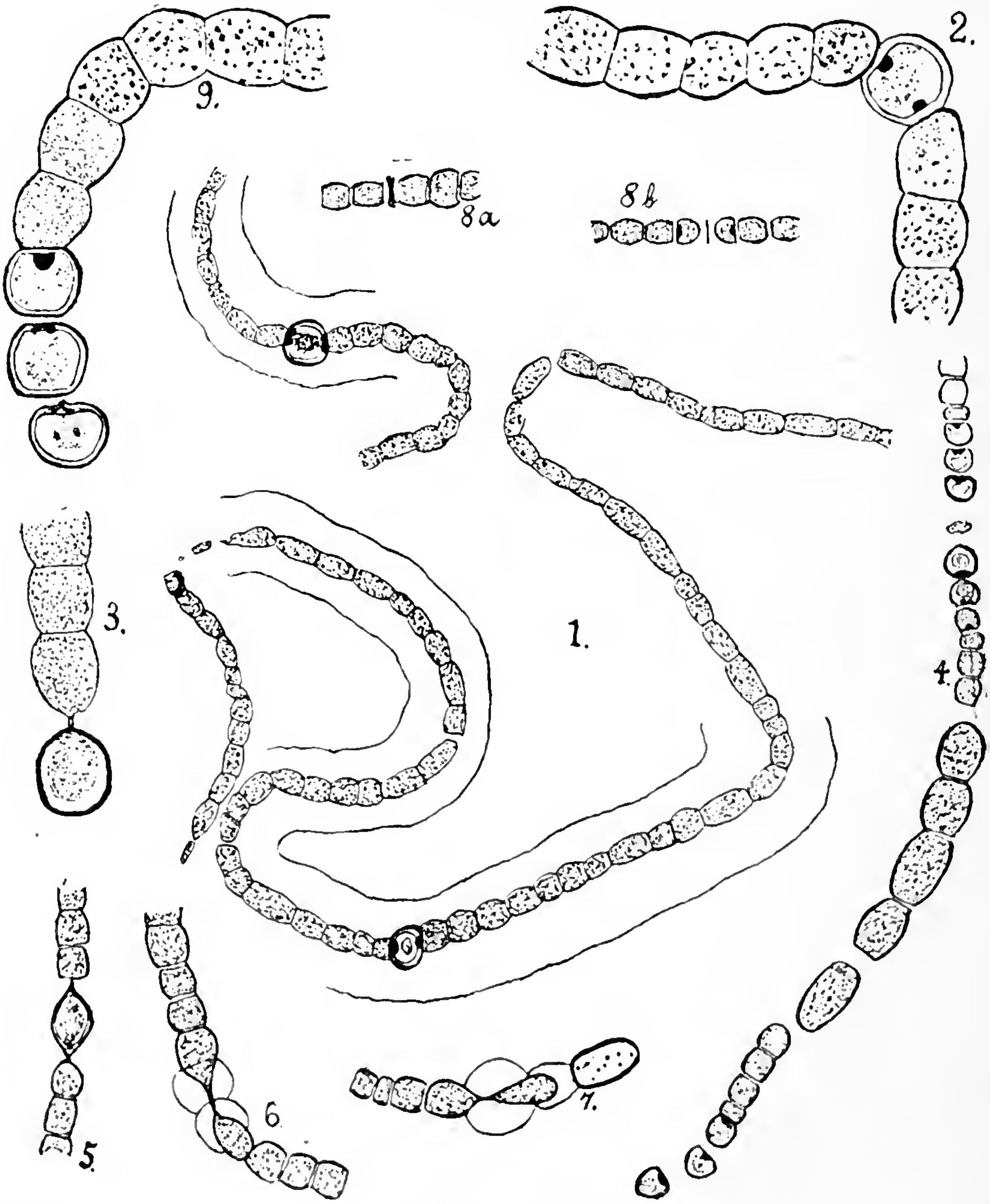
[1] cf. Hieronymus '92, p, 489. Wager 1903, p. 405.

[2] The formation of the spores and their germination will form the subject of the next paper of this series.

Great attention should be paid to those cases, in which spore-formation takes place centripetally *(Sphaerozyga)*; in these cases one would imagine, that the above function would be taken over by the intercalary heterocysts. That raises the question, why does spore-formation in one case commence immediately next to the heterocyst and in another case remote from it? This indicates some influence on the part of the heterocyst, which is as yet quite unknown to us. It is not a point either, which is inherent in the plant; although spore-formation was almost invariably centrifugal in the species under discussion, I met with a few isolated examples of the reverse (cf. Palla '93, p. 545 for *Sphaerozyga*).

Until we have further evidence of the ultimate fate of the



Text Fig. 76.

EXPLANATION OF THE FIGURES,

1. Ordinary vegetative filament, showing intercalary heterocysts and mucilage-sheath. (× 600).
2. Development of an intercalary heterocyst, showing the terminal granules and the uncontracted contents. (× 1500).
3. Terminal heterocyst, showing protoplasmic connection with adjoining vegetative cell. (× 730).
4. Long [filament bounded by numerous terminal heterocysts and forming spores in its central portion. (× 730).
5. Oblong intercalary heterocyst with pointed ends. (× 730).

6 and 7. Protoplasmic connections between the vegetative cells. (× 730).

8*a*. Intercellular substance, excreted between two vegetative cells. (× 730).

8*b*. Devolopment of terminal heterocysts on either side of the same. (× 730).

9. Portion of a filament with three terminal heterocysts, showing two adjoining cells with homogeneous contents, the last formed heterocyst has uncontracted contents and shows the terminal granule. the next shows the lip-like thickenings on either side of the pore, whilst the furthest heterocyst shows the plate-like thickening, the distinct pore and the granules in the contents of the heterocyst (× 1500).

heterocysts, this assumption is without much point. As yet Brand's observation of the germination of the contents stands isolated; its confirmation and the discovery of a similar state of affairs in other genera of the group would in my opinion confirm the spore-like nature of the heterocysts. In these latter structures we probably have the remnants, of what may once have been an important reproductive organ of the Cyanophyceae, whose functions have now in great part been taken over by the spores. Under these circumstances germination of a heterocyst may only take place under very extreme conditions and the failure as yet in confirming Brand's observation may, as this author has himself suggested, merely be due to these conditions not having been attained.

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NOTES ON *TRIGONOCARPUS*, BRONGN. AND *POLYLOPHOSPERMUM*, BRONGN., TWO GENERA OF PALÆOZOIC SEEDS.

By F. W. Oliver.

In a recent paper in the Transactions of the Linnean Society, dealing with the results of a re-investigation of Brongniart's genus of fossil Gymnospermous seeds *Stephanospermum*, the opinion was expressed that a considerable number of the radially symmetrical forms of seeds of Permo-carboniferous age (Radiosperms) possessed the same fundamental type of organisation as *Stephanospermum* itself.[1] The present communication deals with two additional seeds from this provisional group, viz. *Trigonocarpus* and

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[1] Oliver, Trans. Linn. Soc. Second Series, Botany, Vol. VI., p. 392.

Polylophospermum. The specimens were derived from some uncut blocks from the well-known locality Grand 'Croix which had been incorporated in the Williamson Collection many years ago. Through the courtesy of Mr. Smith Woodward, F.R.S., Keeper of the Geological Department of the Natural History Museum, facilities were obtained for preparing microscopical preparations of the seeds. So far as these specimens are concerned, the chief interest lies in the structure of the nucellar wall, and in the possible relations of the nucellus to the integument, and in certain indications which point to the existence of an outer fleshy coat to the testa. The seeds though not new have never been described with that fulness of detail which is desirable in view of the attention that is now being directed to the phylogeny of the Gymnospermous series. In the present note only a few points come up for consideration owing to the incomplete character of the preservation.

Trigonocarpus pusillus, Brongniart,[1] the first of these specimens to be described here, is one of the smallest of palæozoic seeds. It was originally described and figured by Brongniart, but without any elaboration of detail.[2] It is an elliptical seed with beaked micropylar extremity; the testa shews three longitudinal sutures, but is without wings. The hard shell is described as very compact and as shewing a palisade layer ("cellules rayonnantes") at the surface. The dimensions given are 7—8 *mm.* × 4—5 *mm.* The nucellus occupies the interior of the seed and possesses a somewhat tapering or conical pollen-chamber in which pluricellular pollen-grains occur. A single vascular strand enters at the chalaza and expands into a vascular (tracheal) investment of the nucellus, lying in the substance of the latter between the epidermis and the macrospore wall. In transverse section the nucellus is angled in correspondence with the testa, and Renault contributes the additional fact that the angles of the nucellus are attached to the testa along these lines, so that the seed in this respect resembles a *Pachytesta* in miniature.[3]

Our own specimen is 6½ *mm.* in height × 4¼ *mm.* maximum

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[1] The Trigonocarpons first described by Hooker and Binney (Phil. Trans. 1855, p 149) from petrifactions, were regarded by Brongniart (Les graines fossiles, p. 26) as probably belonging to his genus *Tripterospermum*, which differs from *Trigonocarpus* in possessing distinct wings along the sutures. The *Trigonocarpon olivaeforme* of Williamson's 8th Memoir (on the Organisation, &c., Phil. Trans. 1877, p. 213), is doubtless an identical or very nearly related seed. In any case the structural feature used by Brongniart to separate *Trigonocarpus* and *Tripterospermum* seems unimportant.

[2] Les graines fossiles silicifiées, pp. 24, 25 and Pl. B, figs. 1, 2 and 3.

[3] Flore fossile d'Autun et d' Epinac, pt. 2, p. 398.

width. The compact layer of the testa, has a diameter of $\frac{1}{3}$ *mm.*, but its preservation is very imperfect and it is only possible to say that it was probably composed of close-fitting fibrous or sclerotic cells. Peripherally it is overlaid by two or three layers of quite thin-walled, iso-diametric, parenchyma-cells, which, being without external delimitation, very likely represent but a portion of a more extensive tissue (photo. 3, *St.*) These cells could hardly be described as "cellules rayonnantes," so it may be inferred we are dealing here with something not represented in the specimens examined by Brongniart. His "cellules rayonnantes" correspond perhaps to a layer of palisade belonging to the sclerotesta, but not recognised in our specimens owing to the condition of preservation.

The differentiation shewn by the testa in this seed is consistent with the view that *Trigonocarpus* was a drupaceous seed with a hard shell or sclerotesta and an outer sarcotesta.

Since the two sections which we have obtained of this seed are both longitudinal, and neither of them coincides with the plane of any of the three sutures, there is nothing to add to the statements cited above. The identification depends on the form and dimensions of the longitudinal sections.

The *nucellus* tapers slightly towards the apex, which is modified as a pollen-chamber, though no pollen-grains are present in our specimen. The true cavity of this chamber is obviously exaggerated by the contraction of the prothallium, a not unusual feature in seeds of this period.[1] The original floor of the pollen-chamber is shewn at *S*, photo. 3. Above the pollen-chamber, in the more nearly median section (photos. 1 and 3) remains of the base of a nucellar beak are present; but whether this structure was long and tubular as in *Stephanospermum*, or whether on the other hand it ended abruptly, must remain undecided as the distal portion is wanting in our preparation. However this may have been, our seed resembles *Stephanospermum* in possessing a loose flange or collar round the base of the nucellar apical process (photo. 3, *C*). Of several possible interpretations of this collar the most probable is that it represents the epidermis that has become separated from the other tissues of the roof of the pollen-chamber. The causes of its detachment must be sought in the processes of decay that obtained on the death of the seed, combined perhaps, with a local induration of the epidermal tissues and a jamming of the nucellar beak in the micropyle.[2]

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[1] Oliver, loc. cit., Pl. 41, photo 2.

[2] Loc. cit, p. 372.

The epidermis appears to be still *in situ* on the convex sides of the pollen-chamber, but from the level of the pollen-chamber floor and extending almost to the chalaza it is completely separated from the nucellus and in places lies in contact with the lining of the testa (photos. 1 and 2, E). It is quite evident that the gaps on either side of the prothallial region of the nucellus (photo. 2, G) are the result of splitting, and the question arises as to what were the relations of this "epidermis" to the inner surface of the testa, with which it is in contact over part of its course. So far as can be judged from a study of these preparations as well as those of other French seeds of like organization, there would not appear to be any positive evidence for supposing this epidermis to have been adherent to the testa. So that it may be supposed that the nucellus stood originally free within the testa from the chalaza upwards (as in *Lepidocarpon*).

The alternative view that the relations were as in recent Cycads, or, to go back to older types, as in *Lagenostoma* the seed of *Lyginodendron*, is unsupported by observational data and depends on certain theoretical considerations. On this view the collar or flange (photo. 3, C) might be regarded as marking the limits of the free surface of the nucellus, but one difficulty in adopting it rests in the absence of a return-epidermis running to the micropyle. We may pass now to a consideration of the main body of the nucellus. This is occupied by an extensive prothallium of which the preservation is fairly good, though no archegonia are evident in this specimen. The remaining hypoderm of the nucellus, stript of its surface layer, is found cut in tangential sections at places (as at T, photo. 1), and here are seen the tracheal strands, so marked a feature in French Permo-carboniferous seeds. Elsewhere, too, where the wall is cut in a radial direction the tracheal elements are evident, but it is when they can be studied in surface view that they are seen to the best advantage. A portion of the tangentially cut area (T, photo. 1) is shewn enlarged in photo 4. The tracheal strands follow a somewhat irregular course and at places shew anastomoses. The form of the tracheides is rather variable. Whilst some are long and relatively narrow, others, especially such as occur at the junctions or when a strand follows an undulating course, are short and broad. The type of thickening of the wall is a close scalariform easily confounded with spiral. So far as can be judged from the fragmentary specimens, the whole nucellus below its epidermis was ensheathed in a series of anastomosing strands of tracheal elements. The tracheal sheath in this seed consequently differs from that obtaining in *Stephanospermum* on the one hand, where the tracheal mantle is continuous, and from forms like *Aetheotesta* on the other where the strands would appear to run quite independent of one another in a longitudinal direction.[1]

Polylophospermum stephanense, Brongniart,[2] is a long prismatic seed, hexagonal in transverse section and prominently ribbed along the salient angles. Each prismatic face also bears a low median, secondary ridge, whilst the space between the ridges is occupied by a softer tissue.

The seed is some 15 *mm.* in length, and in addition to the long micropylar beak, the testa expands at the apex and base into cupular emergences in such a way as to produce a sort of false chamber at either end of the seed. These chambers enclose respectively the micropylar beak and the pedicel or funicle. The nucellus bears a large distal pollen-chamber, whilst the remains of the prothallium form a vesicle usually open above.

In general our specimens correspond with Brongniart's sketch.[3] Photo 5 shews the seed in fairly median longitudinal section with the micropylar aperture at the summit (*M*). Around it, as at the chalaza also, are shewn the characteristic ensheathing outgrowths or expansions of the testa which form so peculiar a feature in this seed. The internal portions of the seed are contracted away from the testa and include (1) a delicate layer (*E*) probably the remains of the lining of the testa, (2) the nucellus of which the broad, flat-roofed upper portion is the pollen-chamber (*PC*.), whilst the lower tubular body contains the remains of a prothallium here represented by a cellular vesicle open above (*O*), in which feature it precisely recalls Brongniart's figure. In view of the state of preservation of both the specimens no data are available throwing light on the relations of testa and nucellus.

The section adjacent to that figured traverses the pollen-chamber obliquely, whilst lower down it re-enters the body of the nucellus (evidently somewhat displaced) and skims along its wall in a tangential direction. A portion of this tangentially cut tissue is shown considerably enlarged in photo. 10, with the tracheal strands (darker in the photo.) lying *in situ* at many places. In both sections the external limiting layer or epidermis of the nucellus is still in

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[1] B. Renault, Flore fossile d' Autun, &c., p. 274.

[2] Loc. cit., p. 28, pl. C., Fig. 6, 7 and 8.

[3] Loc. cit., pl. C., fig. 6.

position so far as the pollen-chamber is concerned and probably the same is true of the body the nucellus. Its cells are very curiously modified, a large number, especially in the region of the pollen-chamber, projecting as dome-shaped pustules, the summits of which taper into aiguille-like pinnacles. In some instances the limiting membrane of the tapering portion seems to be resolved into a spirally running thread. The whole phenomenon, which is curious and unusual in this position, suggests that an expansion has occurred as a consequence of a mucilaginous break-down of the cell-contents, a break-down that may or may not have been connected with the activity of the putrifactive organisms that everywhere riddle the tissues.

In the nucellus itself the main interest centres in the strands of transfusion tracheides that run in the peripheral tissues. Elements from these strands are shown in photos. 6, 7, 8 and 9. For the most part these tracheides have thickenings which are characteristically scalariform (photos. 7 and 8), occasionally they show a near approach to a reticulated type (photo. 6). Though in the main the strands run longitudinally, at places their course is not quite so regular and indications of cross connections occur as at *A* in photo. 9 (taken from specimen 1492b). Another strand with very distinct scalariform markings on the tracheides is reproduced in photo. 7 (from the same specimen). The tracheides themselves vary in diameter from 12 to 20μ, whilst in length an extreme fluctuation is found from long slender tracheides extending for $\frac{1}{5}mm$ to such as have quite a short truncate outline.

In their distribution in the nucellus the tracheal elements may be traced to a height distinctly above the open sac that represents the prothallium (Photo. 5, *O*), so that it seems probable that they reached to the floor of the pollen-chamber, as in *Stephanospermum*.[1]

In regard to the structure of the testa we have practically nothing to contribute. Everything is consistent with the distribution of harder and softer tissues indicated in Brongniart's sketch of a transverse section of this seed.[2] The hexagonal stone (sclerotesta) is strongly ribbed along the angles, and the harder tissue of which it is composed consists of elegantly reticulated, oblong and pointed elements. The transition from this hard core to the softer enveloping "sarcotesta" would appear to have been gradual. Whilst in specimen 1492 little of the latter remains, in

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[1] Trans. Linn. Soc., 2nd ser. Bot. Vol. vi., p. 369.

[2] Loc. cit., pl. C., Fig. 7.

the other specimen (1487, not figured) a considerable strip of parenchyma remains in position all along one side of the seed and at places it is still in continuity with the stone.

So far then as these fragmentary data go they tend to shew the essential agreement of the two genera of seeds described with *Stephanospermum* and the Trigonocarpons of the English Coal-Measures. The large, broad pollen-chamber, the nucellar tracheides, and the differentiated testa appear to have been group-characters shared by many of the Radiospermic seeds that were so abundant in Permo-carboniferous times. The most difficult point concerns the relations of nucellus to integument, whether the former stands up freely from the latter, or whether these regions are largely coalescent as in living Cycads. The indications on the whole appear to favour the former interpretation, but for quite conclusive evidence we must await the discovery of specimens in which this delicate portion of the seed is more perfectly preserved.

However, leaving this last point open, there are quite adequate data for distinguishing between these forms, which may be provisionally termed the Stephanospermeae, and the Lagenostomas, which doubtless belong to the Lyginodendreae.[1]

As yet no seed of the Stephanospermeae group has been certainly referred to the plant which bore it, though Mr. R. Kidson has recently found impressions of a Radiospermic seed of Rhabdocarpus-type (in the sense of Goeppert and Berger rather than that of Brongniart) attached to a rachis still bearing pinnules of *Neuropteris heterophylla*, one of the Medulloseae.[2] When it is remembered how deeply rooted the suspicion has become associating the Trigonocarpons with the vegetative remains of Medulloseae,[3] and further, that in the last few weeks M. Grand 'Eury from his long investigations at St. Étienne, has added the weight of his authority in favour of the same view,[4] the time when these surmises shall have passed into demonstrated facts should not long be postponed.

In retrospect, the history of the views that have been held as to the origin of the Coal-measure seeds is not devoid of interest. Passing over the older attribution to Palms (which belongs to the

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[1] Proc. Roy. Soc. lxxi., p. 477.

[2] Phil. Trans. B. vol. 197, p. 1.

[3] G. Wild, "On *Trigonocarpon olivaeforme*," Trans. Manchester Geol. Soc., vol. xvi., 1900; D. H. Scott. "On the Origin of Seed-Bearing Plants." Lecture delivered before the Royal Institution of Great Britain, May 15th, 1903, page 11.

[4] "Sur les Rhizomes et les racines des Fougères fossiles et des Cycadofilices," Comptes rendus, tom. 138 (1904), p. 607.

same period as the problem whether Sternberg's *Sigillaria ocellata* was a Cactus or a Euphorbia), we come to the definite identification by Hooker of *Trigonocarpon* as a Gymnosperm seed (loc. cit. 1855). This identification being based on an examination of petrified specimens is of definite and permanent value and differs fundamentally from earlier conjectures depending on casts and impressions.

In 1873, J. S. Newberry published some interesting speculations as to the origin of the numerous forms of *Trigonocarpon* which he had found in the Ohio Coal-Field.[1] He was much inclined to refer them to *Sigillaria* by eliminating the other associated plants as inappropriate. Three years later, when so many fresh seeds had come to light through the agency of Grand 'Eury, Brongniart and Williamson, we find the last-named in doubt as to the plants that could have borne them. "We have recently brought to light such a multitude of seeds that it becomes a matter of extreme perplexity to know on what stems they grew;"[2] and again, "From the number and size of these seeds, it is evident that we have had, at the Carboniferous age, some fifty or sixty species of seed-bearing plants, of which we have as yet been unable to identify the stems and leaves ...... These seeds make an important addition to our knowledge of the flora of the Carboniferous age, but one which requires much further investigation. They will, however, abundantly repay the time and toil bestowed upon them."[3]

In the absence of certain indications as to the origin of these seeds it is not surprising that some latitude should have been taken by Palæo-botanists. Thus, there was the conjecture that the seeds belonged to an upland forest vegetation otherwise unrepresented in the coal. Then, on the definite reference of certain of the seeds to Cordaiteae, a tendency arose to regard them all as belonging to that ancient group of Gymnosperms. Again at one time and another, under various pretexts, occasional seeds have been conjecturally attributed to Gnetaceae and Calamitineae. However from the lack of definite data botanical interest has ceased for twenty years to concern itself with these detached bodies. Nor has this neglect been without compensating advantages. For the vegetative organs of Coal-measure plants have been exhaustively examined meanwhile from an anatomical point of view, and it has been definitely recognised on anatomical grounds alone that many of the fern-like plants of those times exhibited characters

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[1] Geol. Survey of Ohio, Vol. I, Pt. 2, Palaeontology, p 364.

[2] Coal and Coal Plants, a lecture delivered at Glasgow, Nov. 24th, 1875, p. 28.

[3] Loc. cit. p. 29.

transitional to the Cycads. It is these researches[1] that have paved the way for more recent discoveries, and so well that when they came no surprise was evinced. But the value of anatomical characters as indicating affinity has been fully vindicated.

[1] e.g. Williamson and Scott, "Further Observations &c.," Pt. III., Phil. Trans. B, Vol. 186, 1895.

EXPLANATION OF THE PHOTOGRAPHS ON PLATE II.

All the sections have been prepared by Dr. Fr. Krantz of Bonn, from blocks in the Williamson Collection derived from Grand 'Croix. The numbers cited in brackets are the Cabinet numbers of the specimens figured. The photographs were taken by Mr. W. Tams of Cambridge.

Trigonocarpus pusillus, Brongn.

Photo. 1. Nearly median longitudinal section through the seed shewing micropyle and pollen-chamber. The body of the nucellus is occupied by the prothallium. E., stripped epidermis of nucellus; T, portion of wall of nucellus cut tangentially and shewn on a large scale in photo. 4. A more highly magnified view of the apex is given in photo 3. (1486*a*) × 12.

Photo. 2. Longitudinal section from the same seed just touching the side of the pollen-chamber at its insertion. The section is slightly oblique and traverses the axis of the seed about half way up. G, G. gaps on either side due to stripping of epidermis from nucellus. (1486*b*) × about 12.

Photo. 3. Apex of seed in section 1486*a* (photo. 1) more highly magnified. C, collar at base of nucellar beak; S, remains of septum or floor of pollen-chamber; S.T, remains of sarcotesta. × 21.

Photo. 4. Portion of wall of nucellus (T, photo. 1) with sinuous tracheal strand. × 120.

Polylophospermum stephanense, Brongn.

Photo. 5. Longitudinal section. M, micropyle; E, lining of testa; PC. pollen-chamber; O, orifice of prothallial vesicle. (1492*a*) × about 3.

Photo. 6. Reticulated tracheide from nucellus of the same specimen, a scalariform element is seen below. × 275.

Photo. 7. Scalariform tracheides from the nucellus of an adjacent section. (1492*b*) × 265.

Photo. 8. Other scalariform tracheides. (1492*a*) × 280.

Photo. 9. Tracheal strands with junction at A. (1492*b*) × 200.

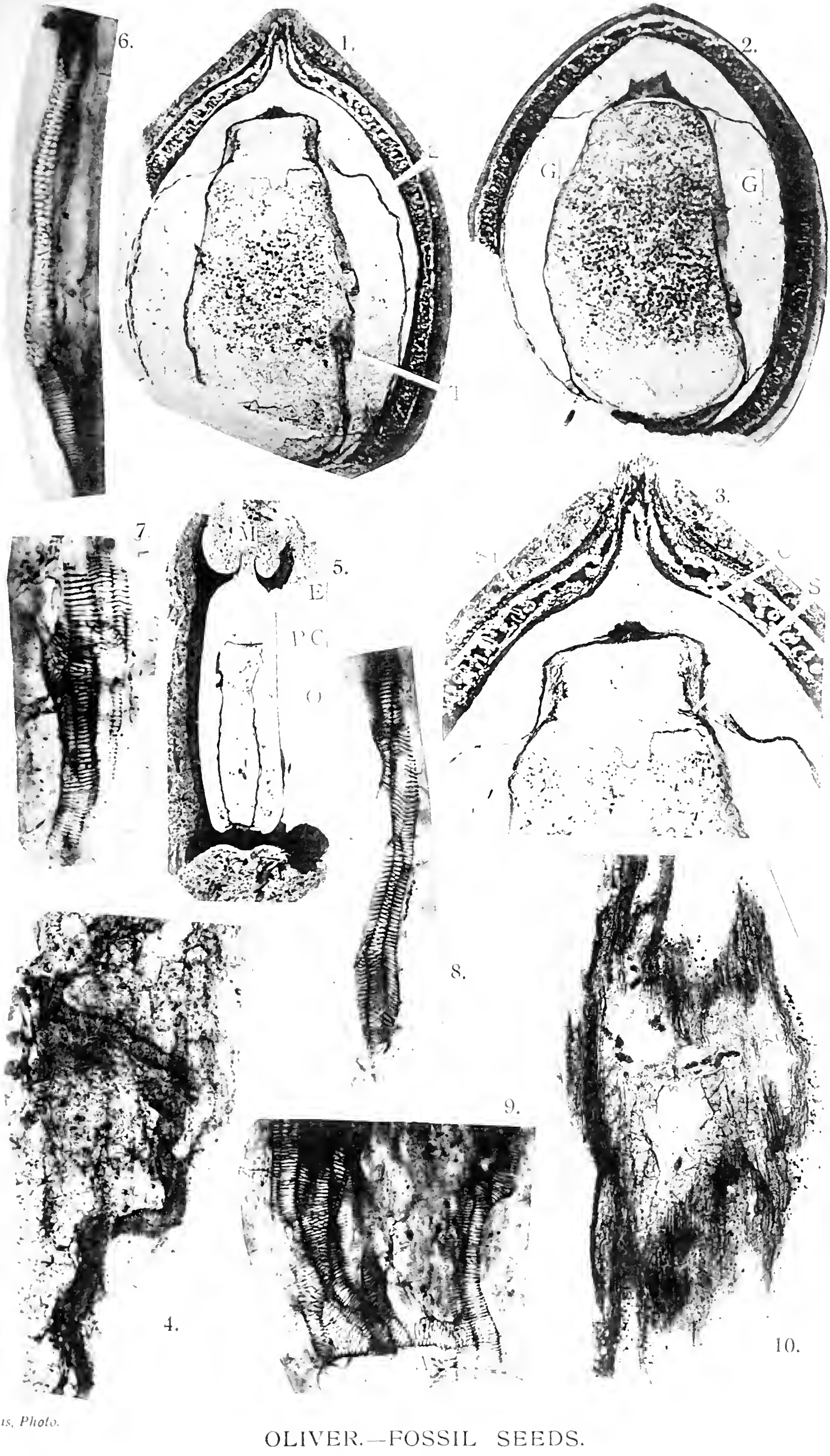
Photo. 10. Fragment of wall of nucellus in tangential section shewing tracheal strands (darker) coursing on the surface. (1492*b*) × 30.

ROOT-STRUCTURE IN THE CENTRAL CYLINDER OF THE HYPOCOTYL.

THE transition-phenomena in the seedlings of *Piper cornifolium* and *Peperomia maculosa* described by Mr. T. G. Hill in the February number of this Journal (Vol. III., p. 46) are of considerable interest to students of the vascular structure of Angiospermous seedlings.

The case recorded as occurring in the former plant is not, however, as he supposes, hitherto undescribed.

It appears to be an extreme example of the type in which a diarch root-stele is formed at or immediately below the cotyledonary node entirely by the two cotyledon-traces, so that the whole or



W. Tams, Photo.

OLIVER.—FOSSIL SEEDS.

nearly the whole of the hypocotyl posseses a central cylinder of typical root-structure. In this type the change from stem-orientation to root-orientation of the vascular elements may take place wholly or partly before the cotyledonary traces have run together to form the stele of the hypocotyledonary axis. It is very common for a considerable portion of this change to occur in the actual bases of the cotyledons, and though its completion is in some cases more or less delayed, the cotyledonary node itself invariably shews the alteration in orientation already begun.

The facts are referred to as *transition-phenomena* as a matter of descriptive convenience, but this designation in no wise involves an expression of opinion as to the morphological value of the cotyledon-traces which have been very variously interpreted. It should for instance be mentioned that the interpretation of the vascular structure of the cotyledonary node arrived at by Sterckx,[1] in accordance with the views of Gravis, an interpretation which supposes that we have to do with a *contact* between cotyledonary bundles and root-stele, is entirely at variance with the language used in the description of this type given above. But this circumstance does not affect the fact that Sterckx's paper contains a particularly full and well-illustrated account of the case in which the "contact" or transition occurs in the cotyledonary node.

This type has been clearly described and figured by Gérard[2] in a number of forms, e.g. *Nigella damascena*, *Argemone grandiflora*, *Fumaria grandiflora*, *Raphanus niger*, *Reseda alba*, and by Sterckx[3] in various Ranunculaceæ. It has also been mentioned by one of us[4] as characteristic of Ranunculaceæ, and again described by Miss Sargant[5] in *Delphinium*, *Nigella* and *Anemone coronaria*.

We have found this type in fourteen genera of Ranunculaceæ, in certain Berberidaceæ, and in every genus examined (twenty-four in all) belonging to the orders Papaveraceæ (including Fumariaceæ), Capparidaceæ, Resedaceæ and Cruciferæ, together forming the cohort Rhœadales of Engler.

The structure in question of course undergoes a certain amount of modification when traced through this wide range of forms.

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[1] Sterckx. Recherches anatomiques sur l'embryon et les plantules dans la famille des Renonculacées. Mem. Soc. Roy. Sci., Liège, sér. 3, t. 2, 1899.

[2] Gérard. Recherches sur le passage de la racine a la tige. Ann. Sci. Nat. Bot., sér. 6, t. 2, 1881.

[3] Loc. cit.

[4] Tansley. Reduction in Descent. NEW PHYTOLOGIST, Vol. I., p. 132, 1902.

[5] Sargant. A Theory of the Origin of Monocotyledons founded on the structure of their seedlings. Ann. of Bot, Vol. 17, Jan., 1903, p. 53, et seq. Diagram IX.

Thus, as already mentioned, there is considerable variation in the precise point at which the different phases of change in the orientation of the xylem and phloem of the cotyledonary bundles takes place, and a further complication is introduced by differences in the level of insertion of the plumular traces. But the essential uniformity of the series of individual cases making up the general type cannot be mistaken.

The extreme case described by Mr. Hill in *Piper*, in which the *whole* change of orientation occurs in the cotyledons themselves, seems to have been previously described and figured only in *Raphanus niger* (Gérard, loc. cit. Pl. 15, Fig. 15). We have, however, found precisely the same case in numerous other Rhœadales; indeed it seems characteristic of that group.

The importance of this simple and striking type of hypocotyledonary structure is sufficiently apparent from its uniformity in the orders named. The temptation to regard it as of phylogenetic importance in relation to the Dicotyledons as a whole, already strong in view of its occurrence in the almost certainly primitive Ranunculaceæ and their allies, is distinctly increased by its discovery in the Piperaceæ. But it is as yet too early to say more.

The striking diversity in the vascular structure of the seedlings existing between many of the Liliaceous genera described by Miss Sargant (loc. cit.) and the similar diversity found by Mr. Wright in different species of *Diospyros*,[1] afford some parallel to the difference described by Mr. Hill between *Piper* and *Peperomia*, a difference which is in striking contrast with the uniformity exhibited by the Ranunculaceæ, Papaveraceæ and Cruciferæ.

The publication of Mr. Hill's full results will be expected with much interest.

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[1] Herbert Wright, Ann. Roy. Bot. Garden, Peradeniya, 1903.

University College,
April, 1904.

A. G. TANSLEY.
E. N. THOMAS.

REVIEW.

ON THE STRUCTURE AND PHYSIOLOGY OF THE CYANOPHYCEAE-CELL.

By F. G. Kohl.[1]

In spite of the efforts of numerous investigators the problem of the structure of the Cyanophyceae-cell remains still unsolved. According to some the Cyanophyceae-cell represents a mass of protoplasm without a nucleus, while others maintain that

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[1] F. G. Kohl; Ueber die Organisation und Physiologie der Cyanophyceenzelle und die Mitotische Teilung ihres Kernes. 8vo. pp. 240., pls. 10. Gustav Fischer, Jena, 1904. Price 20 m.

the cell has a distinct nucleus. There are still others who take somewhat intermediate or conservative views, and find in the Cyanophyceae-cell a rudimentary or undifferentiated nucleus or a nucleus-like structure phylogenetically connected with the nucleus of the higher plants. Professor Kohl, of the University of Marburg, has just published the result of his extensive studies on this subject. The work comprises 240 pages and ten coloured plates. *Tolypothrix*, *Nostoc* and *Anabaena* are the principal forms studied and special attention seems to have been paid to the investigation of *Tolypothrix lanata*. The results of these studies are given in great detail together with a critical discussion of those of previous investigators, under the following heads: 1. Central granules; 2. Cyanophycin granules; 3. Oil; 4. Chromatophores; 5. Glycogen; 6. Membrane and Sheath; 7. Protoplasmic Connections; 8. "Verschlusskörper"; 9. Vacuoles; 10. Chromatic Substance; 11. Heterocysts; 12. Concave Cells; 13. Central Body.

According to Kohl the so-called central body (Zentralkörper) of the Cyanophyceae-cell is a genuine nucleus, and consists of a ground mass which stains relatively faintly, and in which is embedded a certain amount of chromatic substance. It also contains a larger or smaller number of the central granules (Zentralkörner) which have never been found outside the nucleus. The nucleus differs from that of the higher plants in the absence of a nuclear membrane and of nucleoli, and in its peculiar form. It is characterized by numerous pseudopodium-like radiations of various sizes which often reach the cell-wall. The radiating branches are often seen to be drawn into the nucleus after treatment with various fixing re-agents. This is the principal reason that this structure has been overlooked by most previous observers. The chromatophore is not a large hollow cylinder surrounding the central body as Fischer and others maintain, but a very small granule which is found in abundance all through the cytoplasm. The cytoplasm contains, besides chromatophores, cyanophycin granules, oil-drops, glycogen and vacuoles. The chromatophores contain chlorophyll, carotin and phycocyanin. Starch has not been found in any part of the cell, and Kohl takes glycogen for the product of carbon-assimilation since it is formed in the presence of carbonic acid and light, and disappears by darkening. The cyanophycin granules, which are found only in the cytoplasm, represent reserve-albumen, and disappear gradually in the dark. They are found abundantly in the spore and are used up during its germination. The oil-drops are only found in the cytoplasm, being entirely absent from the nucleus or chromatophores. There are two kinds of vacuoles; cell-sap vacuoles and gas vacuoles. The former are relatively rare in the normal vegetative cells, while the latter are found only in certain forms which float on the water surface. The central granules, which are found exclusively in the central body, consist chiefly of an albuminous substance agreeing nearly in its physico-chemical re-actions with the "Voluntanskugel" of A. Meyer. They also contain a substance which takes a blue-black colour when treated with chloriodide of zinc, and pectin substance. The membrane of the vegetative cells and the sheath are not cuticularized, but consist chiefly of chitin, with some cellulose and pectin, while the membrane of the heterocyst is mostly cellulose. All the cells of the *Tolypothrix* filament are found to be connected by protoplasmic threads (Plasmodesmen) through a central pit in the side-wall.

As a strong proof of the nuclear nature of the central body the author describes the process of its division which he calls, without hesitation, mitosis. The description is illustrated by many figures. Before the division the amount of the stainable substance (chromatin) in the central body is found to increase and soon a thick spirem thread is visible. The thread then divides into 4—8 rod-shaped chromosome which arrange themselves near the centre of the cell more or less parallel to the long axis of the filament. The chromosomes divide transversely at the middle and each half passes to the opposite pole to form the daughter nuclei.

At the close of the book some remarks are made on the relationship between the Cyanophyceae and Bacteria. The deeply-staining central part of the Bacteria is considered by Kohl to be homologous with the central body of the Cyanophyceae and represents a true nucleus. He believes, differing from Fischer, that these two groups are intimately related. As an appendix a valuable table containing the most important tests and staining re-actions for the study of the Cyanophyceae-cell is given. A considerable number of staining methods and physico-chemical tests are used in the course of the investigations, both with living and fixed material. But the author does not appear to have made use of microtome sections, a method which has been found very useful in the study of the fine structures of the cell.

The work is indeed a very important contribution to the cytology of the lower organisms and deserves the attention of the zoologist as well as the botanist. K. MIYAKE (Bonn).

R. MADLEY, PRINTER, WHITFIELD STREET, LONDON, W.

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ON SPECIALIZATION OF PARASITISM IN THE *ERYSIPHACEAE*, II.,

BY ERNEST S. SALMON.

IN a previous paper[1] on the present subject I have recorded the results of a series of comparative inoculation-experiments which showed that a far-reaching specialization of parasitism has taken place in certain species of the *Erysiphaceae*.

The present paper gives the results of further inoculation-experiments, carried out during the past summer, in which conidia of the following species were used:—*Erysiphe Graminis* DC., on *Avena sterilis*, *Agropyron repens*, *Poa pratensis*, and *Dactylis glomerata*; *Sphaerotheca Humuli* (DC.) Burr. on *Potentilla reptans*; *S. Humuli* var. *fuliginea* (Schlecht.) Salm. on *Taraxacum officinale* and *Plantago lanceolata*; *E. Cichoracearum* DC. on *Plantago major*; and *E. Galeopsidis* DC. on *Ballota nigra*.[2]

The results obtained seem to show that in every case the form of the fungus used has become specialized into a "biologic form."[3]

Two points of special interest may be noticed here, *viz.* proof of specialization of parasitism in *Sphaerotheca*, a genus in which comparative infection-experiments had not hitherto been carried out; and the formation of perithecia on inoculated leaves of *Taraxacum officinale*, *Plantago major*, and *P. media*.

In all the above cases conidia of the fungus were used. It may be noted here, however, that so far as experiments have been made,

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[1] Beihefte z. Botan. Centralbl. XIV. 261-315 (1903).

[2] The results of a series of experiments carried out at the same time, in which *E. Graminis* on various species of *Bromus* was used, are not included here. These will appear in a paper in the next number of the "*Annales Mycologici*."

[3] A general account of the phenomena met with in the specialization of parasitism in the *Erysiphaceae*, and a definition of "biologic forms," have been given by the writer in a recent number of this journal (p. 55.)

the infection-powers of ascospores are strictly similar to those of the conidia in the case of "biologic forms."[1]

The method of inoculation, &c. adopted in the present experiments was that used in previous work, and has already been fully described[2]. My sincere thanks are due to Professor H. Marshall Ward, F.R.S. for again kindly allowing me to carry out the work in the Cambridge University Botanical Laboratory, where special facilities exist for such work. My thanks are also due to Mr. R. I. Lynch, A.L.S., Curator of the Cambridge Botanic Garden, and to Mr. Hosking, the Foreman, for the trouble and care taken in raising the plants used in the experiments.

I.—Inoculation-Experiments with the Conidia of E. Graminis on Avena sterilis, Agropyron repens, Poa pratensis, and Dactylis glomerata.

The *Oidium* on *Avena sterilis* (see Table 1) proved able to cause full infection when sown on *A. sativa*, the six inoculated leaves all becoming infected. In three experiments nine leaves of *A. pratensis* were inoculated; eight of these were completely passed over, but on the ninth leaf "subinfection"[3] occurred. Inoculation of ten leaves of *Arrhenatherum avenaceum* resulted only in the weak "subinfection" of one leaf. It may be noted that Marchal has recorded[4] that the *Oidium* on *Avena* is able to infect the present species. No details of the inoculation-experiments in which this result was obtained have been published, but the author has kindly informed me that only "subinfection" occurred on the *Arrhenatherum*. In my previous experiments[5] in which leaves of *A. avenaceum* were inoculated with conidia from *Avena sativa*, no infection resulted. No instance is at present known of an *Oidium* possessing the power of causing full infection of species of another genus than the one to which its host-plant belongs. In the present experiments inoculations of

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[1] See *Salmon*, E. S.; in Journ. of Bot., XLI., 159-165, 204-212 (1903); *Marchal*, E.; in Comptes Rendus, CXXXVI., 1280-1281 (1903).

[2] Beihefte z. botan. Centralbl., XIV., p. 266 (1903).

[3] The term "subinfection" is used to denote cases in which inoculation is followed by the production merely of a few scattered conidiophores, which never form powdery *Oidium*-patches, and which usually disappear after a few days. I have already treated of the possible significance of these cases of "subinfection." (See Beihefte z. botan. Centralbl. XIV., p. 270 (1903) and "Annales Mycologici" II, 75 (1904).

Comptes Rendus, CXXXV., 211 (1902).

[5] See Beihefte z. botan. Centralbl., XIV., p. 289, Table XI.

Lolium temulentum, *Festuca elatior*, *Bromus unioloides* and *B. sterilis* were followed by negative results.

The *Oidium* on *Agropyron repens* (Table 2) when sown on *A. tenerum* caused weak but apparently true infection, and apparently true infection when sown on *A. caninum*. No infection followed the inoculation of *A. glaucum* var., and *A. acutum*, although in the case of the latter species one control leaf bore a powdery *Oidium*-patch on the nineteenth day of the experiment.[1]

The *Oidium* on *Poa pratensis* (see Table 3) caused "subinfection" on one of the nine inoculated leaves of *P. annua*, and on one of the three inoculated leaves of *P. nemoralis*. With regard to the "subinfection" of the latter species, the few conidiophores that were produced died away by the twenty-fifth day of the experiment, and although these plants (now flowering) were kept subsequently for over a month by the side of plants of *P. pratensis* covered with powdery *Oidium*-patches, no trace of any further infection occurred on *P. nemoralis*. The present case is of interest as showing that in some cases at least plants on which inoculation is followed by the phenomenon of "subinfection" are, notwithstanding, to be regarded as being for all practical purposes "immune" to the respective fungus, since these plants prove able, under conditions in which virulent infection of the original host-species occurs, to complete their full growth safe from all further attacks.

The inoculation of fifty-three leaves of thirteen species belonging to ten other different genera was attended by negative results only (see Table 3).

The *Oidium* on *Dactylis glomerata* (Table 4) proved unable to infect *Avena sativa*, *Secale cereale*, *Triticum vulgare*, *Lolium temulentum*, *Hordeum vulgare*, and *Agropyron repens*. It is worthy of note, as bearing on the interesting question of the possession of distinctive colour-characters by certain "biologic forms" that the conidia of the *Oidia* on *Poa pratensis* and *Dactylis glomerata* remain permanently snow-white.

II.—INOCULATION-EXPERIMENTS WITH THE CONIDIA OF SPHAEROTHECA HUMULI (DC.) BURR., ERYSIPHE CICHORACEARUM DC., AND E. GALEOPSIDIS DC.

In the experiments with *S. Humuli*, the *Oidium* on *Potentilla reptans* was used (see Table 5). Inoculation of leaves of *P. reptans* was invariably followed by more or less virulent infection, but no

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[1] I am indebted to Dr. O. Stapf for kindly determining the species of *Agropyron* used in the experiments.

signs of infection resulted when conidia were sown on species of *Alchemilla*, *Fragaria*, *Spiraea*, *Agrimonia*, and *Poterium*, on which the morphological species *S. Humuli* occurs.

Hitherto no species of the genus *Sphaerotheca* has been investigated with respect to its adaptive parasitism. The above experiments prove clearly that in *Sphaerotheca*—as in the genus *Erysiphe*—specialization of parasitism has resulted in the evolution of "biologic forms."

The case of the present species is of special interest because *S. Humuli* is the well-known Hop-mildew, and the cause of a disease which—especially in England—annually occasions considerable loss to hop-growers. If it can be shown that the form of the fungus on the Hop, is like that on *Potentilla*, confined to its one host-species, or to species of one genus, the means of extirpating the disease must be sought in destroying all affected wild and cultivated hops, rather than in attempting to remove indiscriminately all the mildewed weeds of a hop-garden.

The *Oidium* of *S. Humuli* var. *fuliginea* (Schlecht.) on *Taraxacum officinale* and *Plantago lanceolata* was also used, and proved in each case to be a specialized "biologic form."

The *Oidium* on *Taraxacum officinale* (see Table 6) produced virulent infection at once when sown on this host-plant, but failed to cause any infection on *Plantago media*, *P. lanceolata*, and the cultivated strawberry (*Fragaria* sp.).

A fact of considerable interest appeared in the two experiments in which the potted plants of *Taraxacum officinale* were inoculated. In the first experiment, on the twenty-first day (September 29) after inoculation, the fungus had produced on the six inoculated leaves many hundreds of perithecia among the still powdery *Oidium*-patches. By the twenty-sixth day (October 4) a great number of ripe dark-brown perithecia—each containing an ascus with ascospores—had been developed on the six leaves. On the twenty-ninth day (October 7) a quite young, scarcely fully grown leaf of the controls was observed bearing a small *Oidium*-patch with a cluster of dark-brown perithecia in the centre. The abundant formation of perithecia on vigorous, quite young leaves, and at places which had previously borne a rich crop of conidia, show that for the production of perithecia other factors must be operative than those assumed by Neger.[1] In the second experiment a for-

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[1] Flora, LXXXVIII., p. 343 (1901). See also the writer's remarks on the present subject in Bull. Torrey Bot. Club, XXIX., p. 19 (1902).

mation of perithecia among powdery *Oidium*-patches took place on the twenty-fifth day (October 5) after the inoculation of the leaves.

The *Oidium* on *Plantago lanceolata* proved unable to infect *P. major* and *Taraxacum officinale* (see Table 7).

In certain experiments with the *Oidium* of *E. Cichoracearum* on *Plantago major* (Table 8) a production of perithecia resulted on inoculated leaves of *P. major* and *P. media*. In the first experiment (No. 199) seedling plants, six weeks old, of *P. major* and *P. media* were used; three leaves of the former species, and two of the latter, were inoculated. On the tenth day after inoculation, numerous patches of mycelium bearing a large number of clustered conidiophores were visible on all three leaves of *P. major*; while one leaf of *P. media* bore a few scattered conidiophores. On the fifteenth day two of the leaves of *P. major* bore young (yellow) perithecia among the powdery *Oidium*-patches; the one infected leaf of *P. media* was now clearly fully infected, and bore a large number of conidiophores. On the nineteenth day numerous perithecia—some turning brown—were observable among the still densely powdery *Oidium*-patches on all the three leaves of *P. major*; on the infected leaf of *P. media* one young (yellow) perithecium was now visible. By the twenty-fourth day the fungus had formed hundreds of brown perithecia on the infected leaves of *P. major*; on *P. media* the single perithecium that was produced had now turned dark-brown, and on examination proved to contain young asci. It may be noted that the single leaf of *P. media* which became infected was a cotyledon, and that although evidently full infection occurred, the number of conidiophores produced was not so great as on *P. major*. In the second experiment (No. 229) two leaves each of seedling plants of *P. major* (seven weeks old) and *P. media* (ten weeks old) were inoculated. On the sixteenth day after inoculation (when the experiment was discontinued) the two inoculated leaves of *P. major* bore large powdery *Oidium*-patches; while one of the leaves of *P. media* was fully infected, and bore numerous nearly powdery patches of conidiophores. In this case the infected leaf was not a cotyledon, but the second or third leaf. In other experiments, in which eight leaves of *P. media* were inoculated, no infection resulted. No infection followed the inoculation of *P. lanceolata*, *Galium Aparine*, and *Eupatorium cannabinum* (see Table 8).

The results of the above experiments show that the form of

E. Cichoracearum on *P. major* is a "biologic form" probably specialized to species of the genus *Plantago*. When sown on *P. media* the result is sometimes full infection, while sometimes the conidia fail entirely to produce any infection. In one case where infection resulted, the fungus produced, after a crop of conidia, a mature perithecium. This is the first instance recorded of the formation of perithecia by a fungus transferred from one host-species to another. We have, it seems, in the present case, an instance of a fungus which has, so to speak, advanced half-way in the process of becoming specialized to a single species of host-plant – in the present case, *P. major;* it is unable apparently to infect *P. lanceolata*, but it can still occasionally fully infect *P. media*, and produce perithecia on this species.

The *Oidium* of *E. Galeopsidis* on *Ballota nigra* (Table 9) proved unable to infect *Salvia verticillata* and *Leonurus Cardiaca*.

TABLE I.

Infection-experiments with conidia of *E. Graminis* on *Avena sterilis.*

Exper. No.	Date, 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
76a	Aug. 2	*Avena sativa*	3	3
93a	,, 13	,,	3	3
76f	,, 2	*A. pratensis*	3	1
76g	,, 2	,,	3	0
93b	,, 13	,,	3	0
76b	,, 2	*Arrhenatherum avenaceum*	3	0
76c	,, 2	,,	4	0
93c	,, 13	,,	3	0
76d	,, 2	*Lolium temulentum*	6	0
76h	,, 2	*Festuca elatior*	3	0
76i	,, 2	*Bromus unioloides*	4	0
76j	,, 2	*B. sterilis*	3	0

REMARKS.

76a. August 7. The inoculated leaves with numerous nearly powdery *Oidium*-patches; controls free. August 20. All three leaves with powdery patches of *Oidium;* fungus spreading to controls.

93a. August 21. Powdery patches of *Oidium* on the three inoculated leaves; controls free. August 26. The three leaves covered with very powdery *Oidium*-patches; fungus spreading to controls.

76f. "Subinfection" only. August 7. A very few scattered conidiophores on one of the inoculated leaves; controls free.

August 20. The little group of conidiophores still visible on the one leaf; no trace of a fungus on any other leaves. September 12. The few conidiophores still persisting, but fungus not increasing and not spreading to other leaves.

76b. "Subinfection" only. August 7. A few scattered conidiophores, three to ten in number, visible on one leaf; controls free. August 20. Fungus died away on the one leaf; no trace of any fungus on the other inoculated leaves nor on the controls. September 12. Leaves of most of the plants infested with *Aphides;* a few small powdery *Oidium*-patches on a few leaves.

TABLE II.

Infection-experiments with conidia of *E. Graminis* on *Agropyron repens.*

Exper. No.	Date. 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
65a	July 25	... *Agropyron repens* ...	3	3
65e	,, 27	... ,, ...	3	3
65b	,, 25	... *A. glaucum* var. ...	3	0
65c	,, 25	... *A. acutum.* ...	3	0
65d	,, 25	... *A. tenerum* ...	3	3
65f	,, 27	... *A. caninum* ...	1	1

REMARKS.

65a. July 31. A few flecks of mycelium with two or three young conidiophores on two of the inoculated leaves. August 2. Well-grown powdery *Oidium*-patches on two leaves, flecks of mycelium with young conidiophores on the third; controls all free. August 5. The three inoculated leaves all virulently infected with numerous powdery *Oidium*-patches; one control plant (of thirteen) now with several flecks of mycelium. August 13. The three inoculated leaves beginning to die; fungus spread to several of the controls.

65e. August 3. Flecks of mycelium on the three inoculated leaves. August 5. The three leaves virulently infected with numerous powdery *Oidium*-patches; controls all free. August 7. The fungus spread to one control. August 13. The three inoculated leaves beginning to die; fungus spread to several of the controls.

65c. No infection of the three inoculated leaves, but on August 13 one leaf of one control-plant bore on the under surface a large well-grown powdery *Oidium*-patch.

65d. Aug. 5. On one inoculated leaf two isolated groups of about ten conidiophores; controls free. August 7. Scattered conidiophores on another inoculated leaf. August 13. Weak infection—slightly more than "subinfection"—of all three leaves; controls all free. September 25. Plants kept in laboratory until this date; a few weak patches of mycelium with a few conidiophores on two of the younger leaves.

65f. August 5. A well-grown patch of conidiophores on the one leaf. August 7. As on August 5; controls free. August 13. Fungus beginning to die away.

TABLE III.

Infection-experiments with conidia of *E. Graminis* on *Poa pratensis*.

Exper. No.	Date. 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
55a	July 17	*Poa pratensis* (young plants in pots)	3	3
88a	Aug. 9	,, ,,	3	3
198a	Sep. 15	,, (seedlings 6 weeks old)	2	2
55b	July 17	*P. annua* (young plants in pots)	3	0
198b	Sep. 15	,, (seedlings 6 weeks old)	2	1
88b	Aug. 9	... *P. nemoralis* ...	3	1
	1902.			
67c	Sep. 10	*Festuca elatior* var. *pratensis*	4	0
67d	,, 10	... *F. arundinacea* ...	4	0
67e	,, 10	... *F. heterophylla* ...	5	0
67f	,, 10	... *Poa annua* ...	4	0
67g	,, 10	... *Lolium perenne* ...	4	0
67r	,, 10	... *L. temulentum* ...	3	0
67h	,, 10	... *Dactylis glomerata* ...	4	0
67i	,, 10	... *Phleum pratense* ...	4	0
67k	,, 10	... *Alopecurus pratensis* ...	4	0
67l	,, 10	... *Avena sativa* ...	3	0
67m	,, 10	... *Hordeum vulgare* ...	3	0
67n	,, 10	... *Triticum vulgare* ...	3	0
67o	,, 10	... *Secale cereale* ...	3	0
67p	,, 10	... *Agropyron repens* ...	3	0
67q	,, 10	... ,, ...	2	0

REMARKS.

55a. July 27. All three leaves infected, and bearing several patches of *Oidium*, some ripe and powdery with little masses of conidia. July 31. The three leaves each with numerous well-developed more or less powdery *Oidium*-patches; controls free. August 2. Powdery patches of *Oidium* on all three leaves; controls still free.

88a. August 20. Numerous very powdery *Oidium*-patches on all the three leaves; controls free. August 26. Fungus spread to several controls. September 3. Fungus spread to all the controls. October 7. All the leaves in the pot white with almost continuous patches of *Oidium*.

198a. September 21. The infected inoculated leaves with numerous vigorous flecks of mycelium bearing groups of young conidiophores. September 25. One leaf covered continuously for a distance of 4·5 cm. with snow-white *Oidium*-patches more or less powdery with masses of ripe conidia; the other leaf with numerous scattered *Oidium*-patches. September 28. Fungus spreading to controls. October 4. Fungus spread to all the controls.

198b. October 4. No signs of infection until this date, when one inoculated leaf bore a little group of five conidiophores; controls free.

88b. August 20. On one inoculated leaf four little tufts of conidiophores, 8–15 in each; controls free. August 26. Conidiophores nearly died away,—no signs of any infection elsewhere. September 3. Fungus quite died away. October 7. No trace of any fungus on any leaves.

TABLE IV.

Infection-Experiments with conidia of *E. Graminis* on *Dactylis glomerata*.

Exper. No.	Date. 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
66a	July 27	... *Dactylis glomerata* ...	3	3
66b	„ 27	... „ ...	3	3
73a	Aug. 1	... „ ...	3	2
78a	„ 4	... „ ...	3	3
66c	July 27	... *Avena sativa* ...	3	0
73d	Aug. 1	... „ ...	3	0
66d	July 27	... *Secale cereale* ...	3	0
66e	„ 27	... *Triticum vulgare* ...	3	0
73b	Aug. 1	... *Lolium temulentum* ...	3	0
73c	„ 1	... *Hordeum vulgare* ...	3	0
78b	„ 4	... *Agropyron repens* ...	3	0

REMARKS.

66a. August 4. The three inoculated leaves with numerous powdery patches of *Oidium*; controls (30) all free. August 7. The three infected leaves each with numerous very powdery snow-white *Oidium*-patches; controls still free. August 11. Fungus beginning to spread to controls.

66b. August 4. The three inoculated leaves all infected, and bearing more or less powdery *Oidium*-patches; controls (33) all free. August 7. The three inoculated leaves with densely powdery snow-white *Oidium*-patches; controls still free. August 11. Fungus spread to several controls.

73a. August 5. A few flecks of mycelium on the three inoculated leaves. August 8. One leaf virulently infected and bearing numerous almost powdery *Oidium*-patches; a few patches of *Oidium* on the second leaf; third leaf not infected. August 11. The two leaves densely covered with very powdery *Oidium*-patches,—the third leaf missed infection; controls still free. August 25. Fungus spread over all the plants in pot.

78a. August 11. Well-grown powdery *Oidium*-patches on all the three inoculated leaves; controls free. August 20. *Oidium*-patches very powdery on all the inoculated leaves, and beginning to spread to controls.

TABLE V.

Infection-experiments with conidia of *Sphaerotheca Humuli* on *Potentilla reptans.*

Exper. No.	Date. 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
149a	Aug. 31	... *Potentilla reptans* ... (young potted plants)	2	2
175a	Sep. 9	... ,, ...	2	2
200a	,, 15	... ,, ...	1	1
218a	,, 21	... ,, ...	2	2
231a	,, 27	... ,, ...	3	3
149b	Aug. 31	... *Alchemilla vulgaris* ... (young potted plants)	3	0
231d	Sep. 27	... *A. arvensis* (seedlings) ...	3	0
175b	,, 9	... *Fragaria* sp. (cult) ...	1	0
231b	,, 27	... ,, ...	3	0
200b	,, 15	... *Spiraea Ulmaria* ...	1	0
149c	Aug. 31	... *Agrimonia Eupatoria* ...	2	0
218b	Sep. 21	.. ,, (seedlings) ...	2	0
231c	,, 27	... *Poterium officinale* ...	3	0

REMARKS.

149a. September 7. The two inoculated leaves covered with patches of conidiophores with chains of ripe conidia; controls free. September 17. Both leaves covered with densely powdery *Oidium*-patches; fungus spreading to control leaves.

175a. September 23. Both leaflets covered with scattered conidiophores; controls free. September 25. Both leaflets with powdery *Oidium*-patches; controls still free. October 5. *Oidium*-patches still powdery on the two leaflets.

200a. September 25. Scattered conidiophores visible over the surface of the inoculated leaflet; controls free. September 29. Powdery *Oidium*-patches on the infected leaflet.

218a. September 30. Virulent infection of both leaves, now covered with powdery *Oidium*-patches; controls free. October 5. The two leaves densely covered with very powdery *Oidium*-patches; fungus spreading to controls.

231a. October 5. The two leaves with scattered conidiophores; controls free. October 7. The two leaves with powdery *Oidium*-patches.

TABLE VI.

Infection-experiments with conidia of *Sphaerotheca Humuli* var. *fuliginea* on *Taraxacum officinale*.

Exper. No.	Date 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
172a	Sept. 8	... *Taraxacum officinale* ... (young potted plants).	3	3
172c	,, 8	... *Taraxacum officinale* ... (young potted plants).	3	3
176a	,, 10	... *Taraxacum officinale* ... (young potted plants).	3	3
172b	,, 8	... *Plantago media* ... (young potted plants).	3	0
172d	,, 8	*Plantago media* (seedlings)	3	0
176b	,, 10	,, (young potted plants)	3	0
172e	,, 8	*P. lanceolata* (young potted plants)	3	0
176c	,, 10	,, ,, ,,	3	0
176d	,, 10	*Fragaria* sp. (cult.) ,,	3	0

REMARKS.

172a and 172c. September 21. Numerous scattered conidiophores at the inoculated places on the six leaves; controls free. September 25. All six leaves virulently infected; numerous vigorous *Oidium*-patches, powdery with masses of ripe conidia; controls still free. September 29. On all six leaves the fungus everywhere passing from the conidial stage into the ascigerous stage; already many hundreds of nearly mature brown perithecia on each infected leaf, as well as young just-formed pale-green ones; controls still free. October 4. All the six leaves with extended patches of crowded dark-brown ripe perithecia, each containing a mature ascus with spores.

176a. September 23. Little scattered groups of conidiophores on all three leaves; controls free. September 25. More or less powdery *Oidium*-patches on all the three inoculated leaves; controls still free. September 29. *Oidium*-patches now very powdery; fungus spreading to controls. October 5. Perithecia in all stages of development—whitish, pale-green, and dark-brown in colour—now visible among the conidiophores on all three leaves.

TABLE VII.

Infection-experiments with conidia of *Sphaerotheca Humuli* var. *fuliginea* on *Plantago lanceolata*.

Exper. No.	Date 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
58a	July 21	... *Plantago lanceolata* ...	2	2
135a	Aug. 24	,,	2	2
58b	July 21	... *P. major* ...	2	0
135b	Aug. 24	... *Taraxacum officinale* ...	2	0

REMARKS.

58a. July 31. Under and upper surfaces of both leaves covered with very numerous scattered conidiophores at the inoculated places; control leaves (five) free. August 3. Both leaves with powdery *Oidium*-patches.

135a. September 8. Numerous scattered conidiophores with chains of ripe conidia at the inoculated places on both leaves; controls free. September 16. Scattered conidiophores on both leaves; controls still free.

(NOTE.—Young potted plants of all the species were used).

TABLE VIII.

Infection-experiments with conidia of *E. Cichoracearum* on *Plantago major*.

Exper. No.	Date. 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
77a	Aug. 4	*Plantago major* (flowering plant)	2	2
199a	Sep. 15	,, (seedlings)	3	3
220a	,, 23	,, ,,	3	3
220e	,, 23	,, ,,	3	3
229a	,, 24	,, ,,	2	2
77b	Aug. 4	*P. lanceolata* (flowering plant)	2	0
220c	Sep. 23	,, (seedlings) ..	3	0
220g	,, 23	,, ,,	3	0
77c	Aug. 4	*P. media* (flowering plant)	2	0
199b	Sep. 15	,, (seedlings)	2	1
220b	,, 23	,, ,,	3	0
220f	,, 23	,, ,,	3	0
229b	,, 24	,, ,,	2	1
77d	Aug. 4	... *Galium Aparine* ...	6	0
220d	Sep. 23	... *Eupatorium cannabinum* ...	3	0

REMARKS.

77a. August 16. Patches of radiating mycelium bearing a few conidiophores on the upper surface of the two leaves at the places of inoculation; control leaves (5) free. August 20. Patches of mycelium bearing more or less powdery groups of conidiophores on both leaves; controls still free.

199a. September 21. Virulent infection of the three leaves, now covered with numerous patches of mycelium bearing a few young conidiophores. September 25. Vigorous groups of conidiophores on all three leaves; controls free. September 30. The three leaves with numerous powdery masses of *Oidium*; a few young (yellow) perithecia visible on two leaves; controls still free October 4. A few perithecia, some brown and nearly mature, on all three leaves, among the still densely powdery *Oidium*-patches.

220a, 220e. September 30. Flecks of mycelium bearing a few young conidiophores on two leaves. October 5. The six leaves densely covered with vigorous extended powdery patches of *Oidium*; controls free. October 10. Large very powdery *Oidium*-patches on the six leaves; on two of the leaves numerous young (yellow) perithecia among the conidiophores.

229a. October 5. Small scattered patches of *Oidium* on the two leaves; controls free. October 10. Large powdery *Oidium*-patches on both leaves.

199b. September 25. A few conidiophores (about 7) visible on one leaf. September 30. One leaf apparently fully infected, and bearing very numerous but rather scattered conidiophores. October 4. On the one infected leaf one young (yellow) perithecium formed among the conidiophores; controls all free. No infection of the other inoculated leaf.

229b. October 10. On one leaf numerous scattered groups of conidiophores and patches of mycelium, in one place nearly powdery with masses of ripe conidia,—apparently full infection; controls free. No infection of the other inoculated leaves.

TABLE IX.

Infection-experiments with conidia of *E. Galeopsidis* on *Ballota nigra*.

Exper. No.	Date 1903.	Species used as Host.	No. of leaves inoculated.	No. of leaves infected.
208a	Sep. 18	... *Ballota nigra* ...	3	3
221a	„ 23	„ (seedlings)	2	2
208b	„ 18	... *Salvia verticillata* ...	3	0
221b	„ 23	... *Leonurus Cardiaca* ...	2	0

REMARKS.

208a. September 25. Patches of mycelium with a few young conidiophores on all the three leaves; controls free. September 28. The three leaves virulently infected, bearing continuous extended snow-white patches of densely crowded conidiophores; controls free. October 5. The three leaves covered with snow-white densely powdery *Oidium*-patches; a few flecks of mycelium now on one control.

221a. October 5. Virulent infection apparent; both leaves covered with snow-white densely powdery *Oidium*-patches; controls free.

THE OCCURRENCE OF *PLEODORINA* IN THE FRESH WATER PLANKTON OF CEYLON.

IN 1893 Shaw[1] described an important new genus of Volvocineae, which, while closely resembling *Eudorina* in type of cell and colony, showed a marked differentiation between the purely vegetative cells and those subserving asexual reproduction (gonidial cells); of the spherical colony of 64 or 128 cells the vegetative cells constitute about one half, occupying the anterior region, whilst the posterior half consists of gonidial cells of 2—3 times the diameter of the former. This Alga was described as *Pleodorina californica*, Shaw. Subsequently a further species—*P. illinoisensis*—was described by Kofoid,[2] in which 32 is the normal number of cells in the colony and in which only four vegetative cells are present, the remaining gonidial cells being up to twice their diameter. Both species have, as far as I am aware, as yet only been found in various localities in the United States.

In the course of a preliminary examination of the Plankton, collected from diverse freshwater areas in Ceylon during September and October of last year, I met with numerous individuals of *Pleodorina californica* in material from two of the tanks of the central low country, *viz.* from tank Periyakulam near Trincomalie, and from Lake Mineri, one of the largest areas of fresh water in the island. The colonies consisted of about 64 cells and were bounded by a well-defined mucilaginous envelope; the gonidial cells were from 15—20μ in diameter, the vegetative cells from 6—9μ. In each of the gonidial cells 3 or more large pyrenoids could be detected, whilst in the vegetative cells only one occurred.

Associated with these typical colonies of *Pleodorina* two other types of colonies were found in both localities. One of these is represented by spherical colonies of 32 cells each, which are about the size of the gonidia of *Pleodorina*, the colonies being indistinguishable from the vegetative form of *Eudorina elegans*. The other type is represented by colonies of 64 cells each, which are about the same size as the vegetative cells of *Pleodorina*. It is possible that these two types represent developmental stages of *Pleodorina*, the 64-celled form arising by the division of all the cells of the 32-celled

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[1] *Pleodorina*, a new genus of the Volvocineae. Botan. Gazette, Vol. XIX., 1894, p. 279—283. Pl. XXVII.

[2] Bulletin Illinois State Laboratory of Nat. Hist., Vol. V., p. 273—293; reprinted in Ann. and Mag. Nat. Hist., Vol. VI., Ser. 7. 1900, p 139—156. Plates V. and VI.

(*Eudorina*-like) type, and subsequently giving rise to the fully differentiated *Pleodorina* by growth of the cells in the posterior half of the colony. Shaw (loc. cit.) has shown, that in the young colonies of *P. californica* the two kinds of cells are not distinct in size. A more detailed study of the material may enable me to arrive at a definite conclusion on this point.

University College, London. F. E. FRITSCH.
May, 1904.

ON THE ARRANGEMENT OF THE VASCULAR STRANDS IN THE "SEEDLINGS" OF CERTAIN LEPTOSPORANGIATE FERNS.

[PRELIMINARY NOTICE.]

FOR some time past the writer has been engaged upon the detailed study of the development of the vascular system in a considerable number of Ferns, mostly belonging to Polypodiaceae. The work has reached a stage of completeness warranting the issue of a preliminary notice, and it is hoped that a full account may soon be published.

The plants examined were representatives of some twenty genera, viz: *Osmundaceae:* Todea. *Cyatheaceae:* Alsophila, Dicksonia. *Polypodiaceae:* Adiantum, Aspidium, Asplenium, Blechnum, Davallia, Doodia, Doryopteris, Lastraea, Lomaria, Nephrodium, Nephrolepis, Notochlaena, Onychium, Polypodium, Polystichum, Pteris. *Hydropterideae:* Pilularia.

On the whole, the earlier stages in the elaboration of the vascular system have been found to be very constant in all the types examined. The primary roots were always of the well-known diarch character, the transition to the solid protostele taking place in the manner rendered so familiar by the work of Gérard, Leclerc du Sablon, Jeffrey and others. At a slightly higher level, we have a few parenchyma cells appearing at the centre of the xylem rod, quickly followed by the differentiation of sieve-tubes, the appearance of the latter elements heralding the immediate formation of the first leaf-trace. The first few leaf-traces do not involve the formation of any real leaf-gaps; in other words the ground-parenchyma does not "encroach upon" the vascular tissue, but merely replaces the xylem and phloem thus temporarily interrupted by the departure of the leaf-trace. Subsequent changes seem to indicate the occurrence of two closely related types in Polypodiaceae.

In the first type, represented by *Doodia aspera*, we find the ground tissue at the leaf-gaps replacing the vascular tissue to such an extent as to divide the latter into two strands, an elementary dictyostelic structure thus being arrived at immediately.

In the second and more common type we find in connection with the fifth, sixth or seventh leaf-trace, the appearance of what the writer proposes to term a "ground-tissue pocket," closely resembling the "endodermal pockets" described by Boodle; the writer believes, however, that the former term more correctly indicates the essential nature of the phenomenon.

The vascular system at the region of such a ground tissue-pocket presents in all essentials the siphonostelic structure regarded by Jeffrey as primitive, but there seems to be no good reason for ascribing such preponderating significance to an arrangement of vascular tissue, such as, in all probability, is merely the expression of the most efficient mechanical distribution of the tissues concerned. The ground-tissue pockets are generally very shallow, the external and internal portions of the ground-tissue becoming continuous at the leaf-gap which occurs at an immediately higher level. Quite exceptionally however, it happens that the pocket may persist through some little distance, and the occurrence of such an internal differentiation of ground-tissue may possibly afford a simple explanation of the highly interesting structure occurring in *Osmunda cinnamomea* as described by Faull.

Polypodium aureum proved to be an extremely interesting plant. Leclerc du Sablon, examining the transitional region of very young plants, describes the occurrence of a central strand of parenchyma in the solid rod of xylem. This has been denied by Jeffrey, and the present work fully confirms the statements of the latter writer. The interest of the plant, however, lies in the fact that after the first two or three leaf-traces (which are formed in a very simple manner), the cauline strand divides directly into two, affording almost a demonstration of the condition of affairs assumed by Van Tieghem as an explanation of his polystelic type. It will be remembered that Miss Ford has described a similar state of things in *Ceratopteris thalictroides*, but the writer hopes to be able to show that the vascular system of *Polypodium aureum* at any rate is essentially phyllosiphonic in character, its true nature being rendered obscure by the occurrence of double, and later, multiple leaf-traces, in conjunction with internodes of somewhat unusual length. The early occurrence of double and multiple leaf-traces was found to be very general in the plants examined.

Examination of the seedlings of Pilularia shows that the development of the vascular system takes place along lines essentially similar to those described for Polypodiaceae. It is hoped that the investigation may soon be extended to Marsilea, a plant of obvious interest in connection with "stelar" questions.

In the present brief note a discussion of the results arrived at is quite impossible. It will be sufficient to add that the early appearance of internal phloem in the primary vascular rod (protostele), and the simple processes involved in the subsequent elaboration of the vascular system of such a plant as *Doodia aspera*, most strongly support the contention of Farmer and Hill that in dealing with all so-called "stelar" questions, we have to confine our attention to the consideration of tissues of two, and only two categories, *viz.*, vascular and non-vascular.

Royal College of Science, S. E. Chandler.
May, 1904.

NITROGEN-METABOLISM ON LAND AND IN THE SEA.

It is evident that the general metabolic cycle in the ocean must be the same as that on land. In both, constituents of the air and of water, together with various inorganic substances are built up by plants into living material. The animals live directly or indirectly on the vegetable substances so produced and at their death are resolved again into their inorganic constituents, with which the cycle of metabolism begins afresh.

The plant-life of the ocean falls easily into two main divisions: one, which includes the larger forms, the fixed algæ, fringing the coasts; the other, the smaller, microscopic, free-floating forms (chiefly Peridineæ, Diatoms and small Flagellata), forming the vegetable plankton. As in the case of trees on land, the fixed algæ, though by far the more striking, are of much less importance from the point of view of the animal food supply of the ocean. It is on the vegetable plankton, as on the less conspicuous herbage on land, that the animal life is mainly dependent.

Owing to the smallness of many of the vegetable plankton organisms the balance between animals and plants, which is an obvious physiological necessity, is sometimes apparently absent. Thus Brandt has found in the bay of Kiel in many cases that more than 60 or 70% of the dried "catch" consisted of animal substance. It is obvious that the "consumers" could not, for the long periods during which this phenomenon was observed, be two to four times as great in quantity as the "producers." As is well-known, however, even tow-nets of the finest miller's silk fail to catch in any quantity the smallest organisms. The disproportion observed by Brandt was probably due to the presence of minute Flagellata which almost completely escaped the net.

It is a well ascertained fact that all plants require a minimum quantity of certain inorganic substances without which life is impossible, and if these are increased to a certain degree, the growth and development of the plants are increased also. As most of the other substances present in the soil are in sufficient quantity to supply all the needs of the plant, the production of plant-substance is practically dependent on the supply of *nitrogen-compounds*. By increase of nitrogen-compounds, as in manuring, the growth of plants can be enormously increased, and by reduction of these compounds the growth is correspondingly lessened. It is clear, then, that the general metabolism on the land as a whole is dependent on the *circulation of nitrogen*.

The sources of nitrogen-compounds in the case of land plants are three in number. In the first case, on the death of existing living beings, the proteid material becomes broken down by putrefaction into ammonia-compounds, which later become converted into nitrites and nitrates. The first process is dependent on the presence of *putrefactive* bacteria and the second on special *nitrifying* bacteria which are found distributed in all soils. Besides this process of the formation of nitrites and nitrates in the soil there is also an opposite process going on in which nitrogen-compounds are broken down into *free* nitrogen, which escapes into the air and is apparently lost. This process is the result of the action of *denitrifying* bacteria. Nitrogen-compounds are also lost to the soil in very large quantities in other ways, for owing to their solubility these substances are washed out of the soil by rain and carried by the rivers to the sea; also by the wasteful process of running sewage into the sea enormous quantities of nitrogen-compounds are continually lost.

It is clear that there must be some natural process or processes by which the stock of nitrogen of the soil can be replenished, for green plants by themselves can make no use whatever of the free nitrogen which surrounds them in such abundance. There are in fact two chief counter-processes which bring again the nitrogen of the air into circulation. A certain amount of combined nitrogen is returned to the soil dissolved in rain, having been produced by electrical discharges in the air.[1] By far the most important method by which the nitrogen of the air is rendered available for plant use is, however, by means of the peculiar symbiosis between certain bacteria and leguminous plants. By this association large quantities of the nitrogen of the air are brought into combination, and on the death and decay of the plant are set free as nitrogen-compounds in the soil.

The circulation of nitrogen in the sea must clearly take place on somewhat the same lines as on land. The constant presence of bacteria in the sea even down to considerable depths (about 400 fathoms in the Atlantic) has been shown by Fischer's work during the German Plankton-Expedition. Any dead animal or vegetable matter would thus soon be broken up into its constituent salts and so rendered available for the use of plants again. The presence also of nitrifying and denitrifying bacteria in the sea has been shown by Baur and Gran. The similarity between the circulation of nitrogen on the sea and on land is so far exact and there is thus a considerable and continual loss of nitrogen which escapes into the air.

There must accordingly be some way of making up for the loss of nitrogen brought about by the denitrifying bacteria and also by the removal from the sea of nitrogenous material, such as fish. Two sources of combined nitrogen have been suggested. First the rivers, which, as mentioned earlier, bring down quantities of dissolved nitrogen-compounds; and secondly the sewage from large towns. Brandt believed that the loss could be largely supplied in this way. Reinke has pointed out, however, that it is very improbable that sufficient could be thus obtained even taking into account the small amount that would be returned in rain; he held the view that probably there were organisms in the sea which could directly use free nitrogen in the same way as the bacteria in association with leguminous plants on land.

The whole problem of the circulation of nitrogen in the sea has entered on a new phase through the discovery (due to Reinke's initiative) by Benecke and Keutner of bacteria in the sea which have actually the power of converting free nitrogen into nitrogen-compounds. The two organisms observed, *Clostridium Pasteurianum*[2]

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[1] According to Knop one litre of rain-water contains about 0.9 mgr. Ammonia.

[2] This organism was first discovered by Winogradsky in soil and was shown by him to live in symbiosis with two other bacteria and to have the power of using free nitrogen.

and *Azotobacter Chroococcum*, were found in the slime at the bottom, and as plankton in the waters, of the bay of Kiel.

These two organisms can live in a medium which contains no nitrogen compound, but they require for their growth and for the absorption of nitrogen a supply of carbohydrate. As this substance is, of course, not to be found in sea-water it seems very probable that it is supplied by some plant with which the bacteria live in symbiosis. The slime in which the *Azotobacter* was found is covered with a fine layer of Diatoms and it seems very probable that the bacteria live in association with them. It was in fact found that the organism would not develop in pure sea-water, but only if the water had been "sown" with a little plankton. The *Azotobacter* was also found growing on the slimy surface of Laminaria, Fucus, etc.; it is very probably in actual symbiosis with them, for such growths are almost of the nature of agar-agar plate cultures, that substance being nothing more than the cell-wall material of certain marine algæ. From the cell-wall material the bacterium could obtain the carbohydrate necessary for its growth, and could, perhaps, supply the alga with the nitrates formed by its activity. In the open sea Reinke suggests that it is possible that the same or similar organisms are to be found actually growing on the surface of the vegetable plankton such as Diatoms and Peridineæ.

The similarity between this symbiosis, if it should be proved to exist, and the well-known one between bacteria and leguminous plants is certainly very striking, and makes the analogy between circulation of nitrogen and the general metabolism on land and in the sea still more close.

The question of the assimilation of free nitrogen in the sea is only one of the important questions in connection with the metabolism of the ocean, our knowledge of which is yet in its infancy. The astonishing difference of proportion which is to be observed between tropical and temperate vegetation on land and in the sea, respectively, is still unexplained. For while the land vegetation is generally much more prolific in the tropics owing to the greater supply of sunlight, higher temperature and greater degree of moisture, yet in the sea the exact opposite is the case. In spite of the greater supply of radiant energy and the higher temperature the plankton and marine vegetation generally is much less in amount in the warmer waters than in temperate and colder regions. Brandt has put forward the interesting suggestion that owing to the higher temperature the denitrifying bacteria are stimulated to great activity, so that a large amount of nitrogen is set free into the atmosphere, thus leaving only a small quantity of nitrogen compounds available for vegetable use in the warmer regions of the ocean. No direct observations, however, have been made on this important matter, and it is very probable that a number of factors combine to bring about the result.

A list is appended of some of the more important papers bearing on the subject discussed.

Baur, E. Über zwei denitrifizierende Bakterien aus den Ostsee. Wissen. Meeresuntersuchungen, Abt. Kiel, VI., 1902, p. 9.

Brandt, K. Über den Stoffwechsel im Meere, Wissen. Meeresunters. Abt. Kiel, IV., 1899, p. 215, VI. 1902, p. 25.

Benecke, W., u. Keutner, J. Über stickstoffbindende Bakterien aus Ostsee. Ber. d. Deutsch. Bot. Ges. XXI., 1903, p. 333.

Gran, H. Studien über Meeresbakterien. Bergens Museums Aarbog, 1901.

Reinke, J. Zur Ernährung der Meeres-Organismen disponibile Quellen an Stickstoff. Ber. d. Deutsch Bot. Ges. XXI., 1903, p. 371.

V.H.B.

REVIEWS.

A Manual and Dictionary of the Flowering Plants and Ferns. By J. C. Willis, M.A., Director of the Royal Botanic Gardens, Ceylon, etc. SECOND EDITION, revised and rearranged in one volume. Cambridge University Press (Cambridge Biological Series), 1904. Price 10s. 6d. net.

WE welcome a new edition of this useful book. The two parts (Part I., "Outlines of the Morphology, Natural History, Classification, Geographical Distribution and Economic Uses of the Flowering Plants and Ferns"; and Part II., a kind of Cyclopædia of classes, cohorts, orders and chief genera) are now included in one compact crown octavo volume, which, owing to the judicious use of small type and abbreviations, only extends to 670 pages, and is not impossible (thanks to the thinness of the paper) for the side-pocket. At the same time a large amount of new material has been added, and controversial matter omitted.

The introductory chapter gains greatly by the addition of a section on the aims of "General Field Work" and of very practical notes on outfit, collecting, preserving and recording, which are evidently the fruit of the author's personal experience in the Tropics.

Part I. has been much improved, and its 200 pages contain an extraordinary amount of varied, accurate, and wonderfully condensed information on morphology, natural history and classification, treated throughout from a thoroughly modern evolutionary standpoint. Many of the sections, indeed, contain by far the best accounts of their respective topics to be found in the English language. The sections on the natural history of the flower, on the principles of classification, on evolution, and on geographical distribution, etc. may be especially mentioned for their excellence. The whole forms a reference work on these subjects that cannot fail to be of great use to travellers and others interested in plants, and also to regular botanical students. At the same time the treatment is too condensed to be altogether suitable as an actual text-book for the student, except under the guidance of a competent teacher.

Of details very few seem open to serious criticism. Surface characters, not internal structure, are the best criterion of the limit between hypocotyl and primary root (p. 37). Enough stress is scarcely laid on the *methods* of reduction prevailing in the evolution of the flower (p. 69), and in this connexion Celakovsky's view of the andrœcium of *Fumaria*, Capparidaceæ and Cruciferæ should at least have been mentioned, since it is much more in accord with the general modern principles of floral morphology than the "chorisis" theory (pp. 70, 333, 416).

The term "ecology" is employed in a wider sense than usual, as equivalent to "natural history" or "Bionomics." We think this a pity, since a word is wanted to express those relations of a plant to its surroundings dependent on what may be called "topographical" factors.

Mr. Willis may be warmly congratulated on what is now a thoroughly excellent book. The typography and get-up are admirable, and the price cannot be complained of when we consider the enormous amount the book contains.

The Classification of Flowering Plants. By Alfred Barton Rendle, M.A., D.Sc, F.L.S., Assistant in the Department of Botany, British Museum, etc. Vol. I., Gymnosperms and Monocotyledons. Cambridge University Press (Cambridge Biological Series), 1904. Pp. XIV. and 403. Price 10s. 6d. net.

It is with pleasure that we open a book in English on systematic botany that is not a translation from the German. Dr. Rendle's aim is "to give the student who has some acquaintance with the rudiments of botany a systematic account of the Flowering plants," the present volume dealing with Gymnosperms and Monocotyledons.

As is indicated in the preface, the view that the Gymnosperms and Angiosperms are really parallel expressions of successful adoption of the seed-habit (which we now have reason to believe was at one time widely spread among the Pteridophytes) has of late years become very widely held; and there now seems very little reason why the Gymnosperms and Angiosperms should be treated side by side in one work.

We suppose the discovery of the "Pteridosperms" is too recent for any account of them to appear in a work which has evidently been in course of elaboration for some time, but it is a pity that the allusion in the preface should be practically contradicted by the categorical statement at the head of Chap. II. on "Spermatophytes" (p. 32): "The Flowering Plants are characterised bythe fo rmation of a *seed*, a structure not found in the remaining groups, known collectively as Cryptogams."

There is an excellent "Historical Introduction" in which the gradual development of the natural system of classification is traced and the different features characterising the various schemes clearly pointed out. We should, however, have been glad of a statement and criticism of Bessey's suggested system (Bot. Gazette, Vol. 24, 1897) and also a fuller account of the relation of Eichler's and Engler's systems to previous ones.

The systematic part of the book is ably and clearly written, and will be most useful to students. It may be doubted whether the rather full details of ovule-pollen and embryo-development are quite in place in a work devoted to classification rather than morphology, especially since they have recently received even fuller treatment at the hands of Messrs. Coulter and Chamberlain; and we should have welcomed instead a discussion and illustration of the principles of classification, a subject far too much neglected in current text-books.

The relation of the Monocotyledons and Dicotyledons is left with the remark that "it is at present impossible to say what is the actual degree of relationship between them." This is no doubttrue, but Dr. Rendle's discussion of this vexed question would have been interesting and valuable, as is his "General Review" of the Monocotyledons, in which we have an able sketch of the characters and probable relationships of the different "series" or cohorts.

It is interesting to note that Dr. Rendle, like Messrs. Coulter and Chamberlain, is not of those who think it necessary to interpret nearly all cases of simplicity in floral structure as examples of reduction.

The illustrations are not very numerous, but have been selected with considerable skill. There are quite a number representing germination-stages (mostly from Lubbock's "Seedlings") and these are very welcome.

We congratulate Dr. Rendle on helping to remove the reproach that our advanced students have to learn their botany from foreign text-books and we look forward for his second part. We could have wished that it might have been found possible to publish the book at a lower price. The high prices of text-books, now that it is absolutely essential for students to have a number of different books dealing with different parts of the subject, is a serious barrier to the wide diffusion of new works, though the publications of the Cambridge Press are by no means the worst examples. We feel sure that the experiment of publication at lower prices would find its justification in greatly increased sales.

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R. MADLEY, PRINTER, WHITFIELD STREET, LONDON, W.

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SPORE FORMATION IN *TORREYA CALIFORNICA.*

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[WITH PLATES III. AND IV.]

I.—INTRODUCTION.

THE genus *Torreya* now consists of four species of restricted distribution, inhabiting respectively Japan, China, Florida, and California. In Cretaceous times it was much more widely spread, being also recorded from Greenland, France, Bohemia, and other districts.[1] Such a history suggests that the genus, which has been comparatively little studied, is an old one, and might be expected to shew primitive characters. The Californian species (*T. californica*, Torrey: syn. *Myristica*, Hooker), known as the Californian Nutmeg, is occasionally cultivated in Great Britain, and there is a fine tree in the gardens at Orton Longueville, near Peterborough, which produces abundant fruit. The Marquis of Huntly has most kindly given every facility for its investigation, and at short intervals, especially throughout the year 1902, consignments of the male and female reproductive organs have been sent to University College by Mr. A. Harding, the head gardener, to whom all concerned with the subject are much indebted.

The peculiar ruminated endosperm of the seed in its second year of development has been described by Professor F. W. Oliver in this journal and he has drawn attention to its similarity to that of certain palæozoic forms. The vascular anatomy of the seeds, which is unique and isolated among recent plants, and also presents analogies with fossil types, has been discussed by the same author in a paper published in the Annals of Botany in 1903[3] and based on

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[1] Pilger. Taxaceæ (Das Pflanzenreich). IV. 5. Leipzig 1903.

[2] F. W. Oliver "On Some Points of Apparent Resemblance in certain Fossil and Recent Gymnospermous Seeds." NEW PHYTOLOGIST, Vol. I. 1902, p. 145.

[3] F. W. Oliver "The Ovules of the Older Gymnosperms." Annals of Botany, Vol. XVII., 1903, p. 451.

a lecture delivered before Section K of the British Association at the Belfast Meeting in 1902. These topics will be dealt with in greater detail in a future memoir. A further contribution to our knowledge of the plant was made by Mrs. A. G. Tansley (Miss Edith Chick), who published an account of the structure of the seedlings in the NEW PHYTOLOGIST for May 1902 (Vol. II. p. 83). The young plants shewed some strikingly primitive characters; centripetal wood was found in the cotyledons, which were lobed and adhered together like those of *Ginkgo* and the Cycads.

A preliminary investigation of the development of the ovules had been carried out by Professor Oliver and Mrs. Tansley,[1] and at the beginning of this year the material collected in 1902 was handed to me in order that the work might be continued. I should like to take this opportunity of expressing my indebtedness to Professor Oliver for his help and encouragement. A number of hand sections had been cut by Mrs. Tansley and these have proved of great value for comparison with my own preparations. Unfortunately the material had not been fixed with a view to cytological work, and the preservation was not good enough to make it possible to work out the history of the reproductive organs except in the early stages where penetration of the fixing fluid is comparatively easy; the present paper thus only deals with the development up to the point of the formation of pollen-grains and embryo-sac. I hope to collect the more important later stages on the spot this summer, and so to carry on the subject further. I have been able to supplement the early stages to some extent by material fixed this year. Flemming's weak solution, followed by Safranin—Gentian-Violet—Orange-G, has proved satisfactory, and Juel's fixative[2] (2 grammes zinc chloride, 2 cc. glacial acetic acid, 100 cc. 40-50°/₀ alcohol) used with Heidenhaim's Haematoxylin has also given some good results. After embedding in paraffin—generally of 52° melting point—the sections were cut to various thicknesses with the microtome, 10μ being usually found most suitable.

II.—THE STAMINATE CONES.

The tree of *Torreya californica* at Orton Longueville bears both staminate cones and ovules. Mr. Harding has kindly given me the following account of their distribution. "The ovules and staminate flowers are not confined to one side of the tree, but for

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[1] F. W. Oliver and E. Chick. On the Morphology of the Seed and Seedling of *Torreya*. British Assoc. Report 1902, p. 814.

[2] H. O. Juel. Ueber der Pollenschlauch von *Cupressus*. Flora, 1904.

some years I have noticed that the ovules are more abundant on the side facing the north, also a sprinkling of male that side as well; but the male is certainly more abundant (four times as many) on the south side. There are less (about half as many) ovules on the south side."

The male cones appear as minute buds in the axils of the leaves on the part of the shoot belonging to the current year, but they may remain dormant for a long time. For instance on one branch gathered this summer no cones had been developed on the parts of the axis corresponding to the years 1904, 1903 and 1902, while a considerable crop of cones occured on the 1901 wood. On another branch ripe cones were found on the 1903 and 1902 wood. At the base of the cone there are a variable number of pairs of decussating bracts. These get more scarious and filmy as we pass up the axis, and one or more of the uppermost pairs have fimbriated margins. During the winter which precedes their ripening season the young cones are completely ensheathed in their bracts, and it is not till the following spring that the bracts separate at the tip and disclose the sporophylls (Figs. 1, 2, 3). A young sporophyll is shewn in longitudinal and transverse section in Figs. 7a and 7b. A very large resin duct runs throughout its length. Four pollen-sacs normally occur on the under side of each sporophyll, but at the apex of the cone there is always some irregularity as to this point, and occasionally an anomalous sporophyll occurs elsewhere. An arrangement which seems not unusual is the termination of the axis in a radially symmetrical sporophyll bearing four pollen-sacs in a peltate fashion. Next below this are placed four sporophylls, each generally bearing only three sacs. In eight nearly ripe cones taken at random, two shewed this arrangement, while in one the terminal sporophyll bore six and in another seven sacs. Four had no terminal sporphyll at all; in two of these the tip was surrounded by four sporophylls each with three sacs, and in two others only one such sporophyll was present, but the remaining members of the whorl had apparently fused and produced a large sporophyll, in one case bearing six, and in the other seven sacs (Figs. 6a—h). The appearance of a nearly ripe cone whose pollen-sacs have not yet dehisced, and the form of its individual sporophylls, are indicated in Figs. 4a, b, c. When the time of pollination is approaching the axis elongates, especially between the uppermost bracts and the lowest sporophylls. In this way the fertile part of the cone is raised out of the bract cup. The four pollen-sacs which were before lying radially, closely appressed to the sporophyll stalk, now turn through an angle of ninety degrees or more and come to occupy a position tangential to the cone, and at right angles to the sporophyll stalk. They each open by a slit along the under side (Figs. 5a, b, c). In the ripe cone the cells forming the wall-layer are stiffened by fine rib-like thickenings (Figs. 15a and 15b).

III.—The Development of the Microspores.

Sections of young male cones collected in November at the stage when they are three or four millimetres long (including the bracts), shew a number of pollen mother-cells already formed, and cell-divisions still taking place (Figs. 1 and 8). The nuclei are large and nearly fill the cells. As a rule they contain two nucleoli, each surrounded by a clear space. Starch is absent from the pollen mother-cells, but present in the cells of the sporophyll. The same distribution of starch apparently persists throughout the winter, as it is still found in cones collected on March 4th. In cones of this date a certain number of the sporogenous cells are in a state of division though these are fewer than in the November material. The structure of the mother-cell nuclei shews no alteration. The tapetum generally consists of one layer of somewhat flattened cells.

By March 19th a considerable change has taken place; the mother-cells have increased in size and become packed with starch, and in most of the cones the nuclei have passed into a condition of synapsis in preparation for the reduction-division. The chromatin collects into a deeply staining ball at one side of the nuclear cavity, and as this takes place simultaneously in all the cells, sections at such a stage present a very striking appearance (Figs. 2 and 9). A single nucleolus can in some cases be demonstrated in the interior of the chromatin mass on treatment with iodine. (Chamberlain[1] mentions that in the case of *Pinus Laricio* he found the pollen mother-cells in the synapsis condition in material collected on April 4th). Nearly three weeks later the division into tetrads takes place. Material collected on April 8th shews the mother-cell nuclei already divided into two or four, and wall-formation taking place (Figs. 3 and 10). Sometimes the spindles of the two divisions could be recognised; but unfortunately the material was badly fixed, and it was impossible to estimate the number of chromosomes. However the contrast between the thick V-shaped chromosomes of the heterotype

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[1] C. Chamberlain. "Winter Characters of Certain Sporangia." Bot. Gaz., Vol. XXV., 1898, p. 125.

division and the longer narrower chromosomes of the succeeding homotype was quite clear in some cases (cf. Figs. 10c and 10d). The two daughter-nuclei which result from the first division are sometimes of great breadth (Fig. 10b). The second division may occur before or after the formation of a wall between the first two daughter-nuclei (cf. Figs. 10a and 10d). The second division commonly takes place in such a way as to give rise to a tetrad, but sometimes the four microspores lie in one plane (cf. Figs. 10g and 10d). Starch is abundantly present in the cells during these divisions. At a slightly later stage the tetrads are found to have separated completely from one another, while only a trace here and there remains of the walls of the mother-cells. Some of the pollen-grains are grouped in pairs, suggesting that they have been produced by a single division of the mother-cell. The nuclei of the tapetal layer are poorly provided with chromatin, and some of the cells are bi-nucleate (Fig. 11). On April 28th nearly all the pollen-grains have dropped apart; they are uni-nucleate and contain a certain amount of starch, though less than was the case three weeks before. It seems probable that the starch was to some extent used up in forming the walls of the pollen-grains, which are thicker than those of the mother-cell, though they have not yet attained to their ultimate thickness. Ten days later the quantity of starch has so much increased as to obscure the nucleus in hand sections.

IV.—THE GERMINATION OF THE MICROSPORES.

By May 31st in material collected in 1902 (Fig. 4c), the pollen-grains have become bi-nucleate, but in 1904 this stage was reached a fortnight sooner. In dividing into two cells whilst still enclosed in the pollen-sac the pollen-grains of *Torreya* agree with those of *Cephalotaxus* and differ from those of *Taxus*[1]. Different stages of the division of the microspore can be found in the same cone—the resting nucleus, the spirem, the spindle, and the two daughter nuclei still united by threads (Figs. 12a—d). At first the two daughter nuclei lie near together in the middle of the cell, and both have a similar granular appearance and contain nucleoli. But one (presumably the generative nucleus) soon retires to one side of the grain, and becomes oval, homogeneous, and Safranin staining, while the other retains its central position and shows no change of structure (Fig. 12e). Coker's figure of the corresponding stage in

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[1] E. Strasburger. Ueber das Verhalten des Pollens und die Befruchtungs-Vorgänge bei den Gymnospermen, Jena, 1892.

Taxodium[1] brings out clearly the contrast between the flattened homogeneous looking generative nucleus, and the larger more openly constructed tube nucleus. Similarly in his paper on *Podocarpus*[2] he mentions that the generative nucleus is always denser than the other free nuclei of the grain; and from his figures it appears that it passes through a flattened period from which it again recovers. The exine cannot be distinguished from the intine until the period when the generative nucleus retires to the side of the cell. More advanced cones gathered on the same day were shedding their pollen-grains. These are angular and tetrahedral and shew three ribs radiating from a point (Fig. 13). On adding water the grains swell with extraordinary rapidity, and in this swelling both cell-body and intine take part. The exine bursts and the grain emerges from it. In the only case in which I actually saw the grain in the act of leaving the exine, it shot out like an orange pip nipped between the fingers. Among his observations on *Sequoia sempervirens*[3] Lawson records that the casting off of the outer wall of the pollen-grains takes place with considerable force. The swelling of the intine is most conspicuous in the equatorial region, and a little papilla is sometimes observed at the end adjacent to the larger cell (Fig. 14). In these ripe pollen-grains the two nuclei are separated by a partition dividing the cell into two very unequal parts, of which the larger is probably the tube-cell and the other the generative cell. The wall between is thin, and either straight or convex towards the larger cell. The mature pollen-grains contain little or no starch, whereas a few uni-nucleate grains among them, whose intines are much thinner, still contain a good deal of starch. As the loss of starch and the thickening of the intine take place synchronously it seems reasonable to infer that the starch is used up to form the thickened wall. If I may hazard a conjecture as to the use of the thick intine, I should like to suggest that by its mucilaginousness it may anchor the grain to the tip of the nucellus, and also it may conceivably serve as a store of reserve carbohydrate, upon which the germinating pollen-grain can draw when it is manufacturing its long cellulose tube.

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[1] W. C. Coker. On the Gametophytes and Embryo of *Taxodium*. Bot. Gaz., Vol. XXXIV., 1903, pp. 1 and 114.

[2] W. C. Coker. Notes on the Gametophytes and Embryo of *Podocarpus*. Bot. Gaz., Vol. XXXIII., 1902, p. 89.

[3] A. A. Lawson. The Gametophytes, Archegonia, Fertilisation, and Embryo of *Sequoia sempervirens*. Annals of Botany, Vol. XVIII., 1904, p. 1.

V.—The Phylogenetic Significance of the Male Gametophyte in Gymnosperms.

In *Torreya californica* there are no signs of any sterile prothallial cells, either in the ripe pollen-grain or during its development. Coulter and Chamberlain[1] point out that these structures are usually very ephemeral, and may easily escape observation, and they conclude, "We venture the opinion, therefore, that one or two vegetative cells, more or less evanescent, will be found to be of common occurrence among Gymnosperms." Since this was written, however, Coker has carefully looked for these cells in *Taxodium*[2], *Cupressus* (4 spp), *Taxus baccata* and four vars., *Juniperus* (2 spp), *Chamæcyparissas* (5 spp), *Callitris* (1 sp.) *Cryptomeria japonica*, and *Thuja orientalis*, and reports their absence in all these cases[3]. Lawson[4] also states that the most searching examination failed to reveal a trace of prothallial cells in *Sequoia sempervirens*. I think therefore that we are justified in assuming that male prothallial cells are entirely absent in a certain number of Gymnosperms. Their presence or absence is a character to which we should be naturally inclined to attach some phylogenetic importance. In the case of ferns Heim[5] has brought forward evidence to shew that indications as to the affinities of the different groups may reasonably be looked for in the gametophyte as well as the sporophyte generation. Fern prothalli are notoriously susceptible to the influence of external conditions, and Heim carried out cultural experiments to determine what characters were dependent on such conditions, and hence were of no phylogenetic value. After eliminating these he found that growth by an apical cell or a marginal meristem, the presence or absence of hairs, and the mode of dehiscence of the antheridia, were characters which were constant throughout groups which would be regarded as related on the evidence of the sporophyte generation alone. It is clear that the characters of the gametophyte can only be used with great caution as indications of affinity. But there is nothing peculiar about this; the question of how to distinguish ancestral from adaptive characters is one that confronts us in every branch of the study of structure. It is the crux of the whole situation, and if it could be eliminated morphology would lose at once both its difficulty and its charm. In the present state of our

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[1] Morphology of Spermatophytes, Part I., New York, 1901.

[2] loc. cit.

[3] Note in Bot. Gaz., May, 1904, ref. to Science, N.S., 3: 424, 1904.

loc. cit.

[5] Carl Heim. Untersuchungen über Farnprothallien. Flora, 1896.

ignorance general criteria are not available, so each case that arises must be dealt with on its own merits. In certain instances, as for example in those *Lycopodium* prothalli which have taken to a saprophytic mode of life, ancestral characters are obviously swamped by adaptive ones. Even in less extreme cases the paramount necessity of securing sufficient nourishment for the egg, and a satisfactory start in life for the young embryo, must tend to overrule vestigial characters in the female organ. Thus it is in the antheridia, or in the male gametophyte if the prothallia are diœcious, that we should be disposed to expect a more marked survival of ancestral characters, as natural selection would have operated less actively in eliminating them. This is borne out by the fact that Heim finds structural differences between the antheridia of various groups of ferns, which are not paralleled by corresponding differences in the archegonia.

It is now generally believed that the Gymnosperms have developed from fern-like ancestors, and hence we must suppose that the very ancient primæval ancestors of our modern Conifers had a well-developed prothallus which has since undergone a process of reduction. In all the Gymnosperms of the present day the female prothallus survives in the form of the endosperm—retained, presumably, because it could be turned to account as a food-tissue. But the male prothallus is extremely reduced, consisting at the most of two cells, or is even absent altogether. The most archaic types now surviving are those in which we should expect to find the clearest traces of such a prothallus. The Cycads and *Ginkgo*, which are recognised on other grounds as comparatively primitive types, possess respectively one and two prothallial cells. That traces of the prothallus should survive in these plants is just what we should expect, but we further find that the possession of two prothallial cells is a character shared by the Abietineæ, perhaps the most highly modified of the Coniferales, and also by *Podocarpus*, a genus referred to the Taxaceæ and thus very remote both from the Abietineæ and from *Ginkgo!* Two families among the Pinaceæ (Taxodieæ and Cupresseæ) and one among the Taxaceæ (Taxeæ) are alike in shewing no trace of a male prothallus. So at first sight the presence of prothallial cells seems to be a capricious character, appearing unaccountably in groups which we are forced on other grounds to regard as standing at different morphological levels. I think, however, that we are here dealing with the case of an ancestral character obscured by an adaptive one. Somewhere in the course of the evolution of the Gymnosperms a stage must have been reached when the microspore as a whole, instead of the naked sperm, assumed the duty of journeying to the archegonium. Before that time the male prothallus, even if it was of little use, would probably have survived, as the possession of it would not have been an active disadvantage to the plant. But as soon as wind transference came into play, lightness became a very important quality in the microspore, and every superfluous structure would be rigidly eliminated by natural selection. The pollen-grains would have to be carried by the wind even to secure *self*-fertilisation, and the lighter the grain, the greater would be its chance of being conveyed to a distant tree and so effecting *cross* fertilisation and the production of a vigorous offspring, which would be more likely to succeed in the struggle for existence, and to perpetuate the characters of its parents. In this way we may suppose the pollen-grains of the Taxodieæ, Cupresseæ and Taxeæ to have reached their present state. *Ginkgo* and the Cycads are at a lower level, and we may imagine that they represent the condition through which the Taxodieæ, etc. passed before their male prothalli vanished entirely.

But if this is the explanation of the loss of the male prothallus in the Taxodieæ, Cupresseæ and Taxeæ, how is its survival in the Abietineæ, and the distant genus *Podocarpus*, to be accounted for? I should like to suggest that in both these cases there is an actual correlation between the survival of the prothallus and the *winged* character of the pollen-grain. The pollen-grains of the Taxodieæ, Cupresseæ and Taxeæ are not winged, while the specific gravity of those of the Abietineæ and of *Podocarpus* is reduced by bladder-like swellings of the exine. The superior buoyancy thus produced would make any minute difference in weight of very little importance, and so natural selection would not have come into play to eliminate the vestigial sterile cells in the pollen-grain. One genus of the Abietineæ, *Tsuga*, has wingless pollen-grains, but I have not been able to learn whether they contain any sterile cells. It certainly seems probable that we should find prothallial cells here, as in the other Abietineæ, for in the case of a single wingless member of a family characterised by wings we must suppose that the absence of wings is not a primitive trait but a comparatively modern case of reduction, and hence it will not be surprising if there has not been time for the loss of the prothallial cells.

In the temperate regions of the northern hemisphere *Pinus* is the dominant genus among the Conifers, while in the south its place is taken by *Podocarpus*. May we perhaps attribute the conspicuous success of these two genera, whose structure is otherwise so diverse, to the winged pollen-grains, which would, in the matter of cross-fertilisation, give them a distinct advantage over their wingless competitors?

VI.—The Female "Flowers."

The ovules of *Torreya californica* occur on the shoots of the current year, especially near the base. In the axil of a foliage leaf an extremely short branch arises bearing two bracts, B and B′, which stand to right and left. In the axil of each an ovule is produced with two pairs of bracts below it. The first pair, b_1 and b_2, is placed at right angles to B and B′, whilst the second pair, b_3 and b_4, is parallel to B and B′. Between the ovules a rudimentary growing point occurs which, as an occasional abnormality terminates in a third ovule[1], or produces an extra bract. Sections of a bud gathered on December 1st, disclosed young ovules already shewing the rudiment of nucellus and integument (Fig. 16). There is not much change during the winter, but in material collected on March 4th, the two bracts B and B′ are discernible (Figs. 17a and b). Examination of ovules collected a fortnight later shews that by this date the bract of the lower pari remote from the axis has appeared, while its fellow has not yet developed (Fig. 18). Before the end of April the integument has over-topped the nucellus and the arillus has begun to appear (Fig. 20b). Material collected on May 8th, and cut longitudinally shews the arrangement of the bracts and the different parts of the ovule very clearly (Figs. 21a, b, c). The little swelling between the ovules which represents the growing point of the dwarf shoot does not occur exactly in the median plane of the ovules, and so is not visible in the section drawn, but its position as seen a few sections further on is indicated by a dotted line. The embryo-sac mother-cell has not yet become distinguishable from its neighbours.

At the time of pollination, three or four weeks later, a drop of liquid exudes from the micropyle, and in this the pollen grains are caught.

VII.—Development of the Megaspore.

In material collected on May 21st, the embryo-sac mother-cell is visible for the first time (Figs 22a, b, c). The nucleus is large and resting, and the protoplasm packed with starch, while the

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[1] A third ovule in this position is recorded as an abnormality in the related *Torreya nucifera* by Strasburger (Angiospermen und Gymnospermen, 1879).

surrounding cells are starch free. The nucellus does not as a rule shew a very definite arrangement of the cells in linear series ; the most distinct indications of such an arrangement occur in a section cut at right angles to the plane in which the ovules lie. The grouping of the surrounding cells is shewn in Fig. 23. The mother cell is not accompanied by a large-celled tapetum, such as that found by Coker in *Taxodium*, but the cells immediately round it are distinguished by dense contents and rather conspicuous and active looking nuclei. The mother-cell was much further advanced this year than in 1902, for among the material collected on May 17th, there were some mother-cells in synapsis (Fig. 25). This stage was not reached in 1902 until three weeks later. Coker[1] describes and figures synapsis in the mother-cell of *Taxodium* at a corresponding stage, and Juel[2] figures what appears to be a condition of rather incomplete synapsis in the embryo-sac of *Larix sibirica*. The majority of the embryo-sac mother-cells in synapsis which I have seen had been fixed in alcohol. I have only examined one mother-cell in this condition fixed in weak Flemming and one fixed in chromic acid. In both these cases I have found a delicate brush of fibrils in the protoplasm at the basal end of the cell (Fig. 25). So I should imagine that its absence in the alcohol material was due to imperfect preservation. In the case of *Larix sibirica* Juel describes a fibrous structure in the protoplasm occurring as a rule at the upper end, and once at the base of the embryo-sac mother-cell. But it differs from the structure in *Torreya* to which I refer, in not occurring until the stage when the nuclear thread is beaded, and also in being in the form of a meshwork and not a brush. In *Taxodium* Coker[3] mentions the occurrence of denser fibrous areas which he regards as of the same nature as those in *Larix*, but in his figures they appear to be rather granular than fibrous, and hence differ still further from the appearances in *Torreya*. When the embryo-sac mother-cell is in synapsis it is packed with starch, which is absent in the surrounding cells. In material collected on June 1st, 1904, which shewed the mother-cells at various stages of development, three of them had nuclei with beaded threads, and so were probably at a stage of development intermediate between the synapis and the reduction spindle (Fig. 26). On June 24th, 1902, a fortnight after the occurrence of the

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[1] loc. cit.

[2] H. O. Juel. Beiträge zur Kenntniss der Tetradentheilung. Pringsheims Jahrb., Bd. 35, 1900 p. 626.

[3] loc. cit.

synapsis stage, the spindle of the reduction division was found in two cases (Figs 27a and b, and 28); two were also found in material collected on June 1st, and one on June 7th, of this year. I have not been able to count the chromosomes with any certainty, partly because in almost every case the spindle appears in two successive sections. At this stage there is a mass of starch at each end of the mother cell (Fig. 28). The next step in the development is represented in Fig. 29 which shews the two daughter nuclei separated by a wall, though still connected by threads traversing the partition. Starch is present in both the daughter cells, which are of unequal size. Both cells divide again, but the division of the lower cell precedes that of the upper one. Fig. 30 shews a stage at which the division of the lower cell is completed while the upper cell is still in the spindle condition. (In the early division of the lower and larger cell and the obliqueness of the upper spindle *Torreya* resembles *Larix*.)[1] In this case abundant starch occurred in the lowest cell and in the cell containing the spindle, while it was almost or entirely absent from the middle cell. A later stage is that drawn in Fig. 31. Four potential megaspores have been produced; the imperfectly preserved wall separating the two upper ones is nearly longitudinal, but in the next section to that drawn the lower part of it was seen to be more oblique. Here starch was again absent in the second cell from the base, though abundant in the other three cells. The absence of starch in one of the four spores seems curious until we recollect that at the period of the reduction spindle it was massed at the two ends of the mother-cell, and hence it is now naturally confined to the basal spore, and the two sister spores which occupy the upper end of the mother-cell. The starchless cell is peculiar in having a tuft of fibrils extending laterally from its nucleus to one of the side walls. The basal spore—the embryo-sac—now increases greatly in size, and the three cells above it are flattened by its growth, their nuclei becoming homogeneous and darkly staining. This stage is illustrated by Fig. 32 which was sketched from an ovule gathered on June 22nd of this year. The three ovules from which Figs. 30, 31, 32 were drawn are the only ones in which I have actually observed the division into four of the megaspore mother-cells.

In *Taxodium*, Coker[2] records the division of the megaspore

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[1] H. O. Juel. Beiträge zur Kenntniss der Tetradentheilung. Prings Jahrb., Bd 35, 1900, p. 626.

[2] loc. cit.

mother-cell into three cells only. In the case of *Sequoia sempervirens*, Lawson[1] has only been able to observe a single division, although Shaw[2] had previously stated that four spores occurred in this species. *Torreya californica* resembles the nearly related *Taxus baccata*,[3] as well as *Larix sibirica*[4] and *Pinus Laricio*[5] in the fact that the megaspore mother-cells give rise to four spores of which the lowest undergoes further development and is the functional embryo sac.

In one ovule, collected on July 6th of this year, I have observed two prothalli, which appear to have been formed from different mother-cells. This is the only case I have so far seen in which there was any sign of the existence of more than one mother-cell.

VII.—SUMMARY.

(A.) MICROSPORE DEVELOPMENT.

The male cones of *Torreya californica* pass the winter in the mother-cell condition. The synapsis state is found about three months before it occurs in the embryo-sac mother-cell. Starch is absent in the mother-cells through the winter, but appears when the nucleus is preparing for the reduction division, and does not disappear again until the pollen grains become bi-nucleate.

No prothallial-cells are cut off, and it s suggested that when such cells occur in the pollen grains of the higher gymnosperms, their survival is correlated with the presence of bladders in the exine, which favour wind transference. In cases such as *Torreya* where no bladders have been developed, it is supposed that the loss of the vestigial prothallus has been brought about to secure lightness of flight.

(B.) MEGASPORE DEVELOPMENT.

The ovules with nucellus and integument already differentiated are found in the winter buds. By the end of April the integument has over-topped the nucellus, and the arillus begins to appear. The embryo-sac mother-cell is first distinguishable in the latter half of May. A well-marked synapsis stage precedes the reduction

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[1] loc. cit.

[2] W. R. Shaw. Contribution to the Life-history of *Sequoia sempervirens*. Bot. Gaz. XXI., p. 332.

[3] E. Strasburger. Anlage des Embryosackes und Prothalliumbildung bei der Eibe. Festschrift zur siebzigten Geburtstage von Ernst Haeckel. Jena 1904. Reviewed, Bot. Gaz., June, 1904.

[4] Juel. loc. cit.

[5] Coulter and Chamberlain. loc. cit.

division which is found in different years on days so far apart as June 1st and June 24th. The mother-cell divides into four, of which the basal cell is the embryo sac. Starch is present when the mother-cell first becomes distinguishable from its neighbours, and it has not disappeared when the division into four is completed.

EXPLANATION OF PLATES III. AND IV.

ILLUSTRATING MISS AGNES ROBERTSON'S PAPER ON "SPORE-FORMATION IN *TORREYA CALIFORNICA*."

PLATE III.—THE STAMINATE CONE.

Fig. 1. Staminate Cone in winter resting condition enclosed in its bracts (× 2) (see Fig. 8.) Collected November 10th, 1902.

Fig. 2. Staminate cone collected March 19th, 1902, shewing bracts beginning to open. (× 2) (cf. Fig. 9.)

Fig. 3. Staminate cone collected April 8th, 1902 (× 2). (See Fig. 10).

Fig. 4a. Ripe staminate cone collected May 31st, 1902 (× 2.) The sporophylls are now fully exposed. At this stage the pollen grains are bi-nucleate. (cf. Fig. 12c.)

Fig. 4b and 4c. Enlarged dorsal and ventral view of a single sporophyll with four pollen sacs from the cone drawn in Fig. 4a.

Fig. 5a. Dry cone which has has shed its pollen grains (× 2). Collected June 1st, 1904.

Figs. 5b and 5c. Enlarged dorsal and ventral view of a single sporophyll from the cone drawn in Fig. 5a. The pollen sacs have opened by slits on their lower sides, and the whole sporophyll and the sacs have shrunk considerably.

Figs. 6a—6h. Diagrams of the tips of eight cones collected on May 31st, 1902 (see Fig. 4a) viewed from above, shewing the different arrangements of the sporophylls at the cone apex.

Fig. 7a. Longitudinal (nearly radial) section of a young stamen (× 21). Cone collected March 4th, 1902. (*r*, resin duct, ; *t*, tapetum ; *p*, pollen sac.

Fig. 7b. Transverse section (tangential to the whole cone) of a young stamen (× 21). cone collected March 4, 1902. Lettering as in Fig. 7a.

Fig. 8. Pollen mother-cells in winter resting condition (× 600). Collected November 10th, 1902. (c.f. Fig. 1).

Fig. 9. Pollen mother-cells in synapsis (× 600). Material collected March 19th, 1902. (c.f. Fig. 2).

Figs. 10a—10g. Tetrad division of the pollen mother-cells (× 600). Material collected April 8th, 1902 (c f. Fig. 3).

Figs. 10a and 10b. Pollen mother-cells shortly after the reduction division.

Fig. 10c. Three chromosomes from a polar view of one of the two daughter nuclei resulting from the reduction division.

Fig. 10d. Second division occurring in a pollen mother-cell before the two first two daughter nuclei are separated by a wall. Note the contrast between their chromosomes and those shown in Fig. 10c.

Fig. 10e. Four daughter nuclei formed, but mother-cell still only divided into two. In this case, as in Fig. 10d, the four daughter nuclei lie in one plane.

Figs. 10f and 10g. Cases of tetrad formation.

Fig. 11. Section of d part of a pollen sac from a staminate cone gathered April 22nd, 1904 and kept in water until April 28th (× 600) *w*, wall layer; *t*, tapetum. The pollen-grains are still grouped in tetrads, double tetrads, or pairs. Occasional traces of the walls of the mother-cells are seen.

Figs. 12a—12e. Different stages in the division which separates the generative nucleus from the tube nucleus. (× 600). Material collected May 17th, 1904.

Fig. 12a. Resting nucleus.

Fig. 12b. Nucleus in spirem.

Fig. 12c. Obliquely cut spindle.

Fig. 12d. Two similar daughter nuclei still united by threads.

Fig. 12e. Differentiation of the two nuclei (tube nucleus and generative nucleus) *e*, exine; *i*, intine.

Fig. 13. Ripe pollen-grain examined dry, shewing triradiate ribbing. (× 600). Material collected May 17th, 1904.

Fig. 14. Ripe pollen-grain examined in aqueous iodine. (× 600). *e*, cast off exine; *i*, swollen intine.

Fig. 15a and 15b. Transverse and tangential views of the wall of a ripe pollen sac to shew ribbing of outer layer of wall. (× 140). Material collected May 17th, 1904.

Plate IV.—The Ovules.

Lettering throughout:—*n*, nucellus; *i*, integument; *l*, leaf subtending ovules; *B* and *B'*, bracts in whose axils flowers arise; *b* and b_2, lower pair of bracts at base of ovule; b_3 and b_4, upper pair of bracts at base of ovule; *r*, resin passage; *a*, arillus; *e.m.c*, embryo-sac mother-cell.

Fig. 16. Longitudinal section (radial to the fertile shoot) of an ovule from a winter bud. (× 21). Collected December 1st, 1902.

Fig. 17a. Longitudinal section (radial to the fertile shoot) of an ovule collected March 4th, 1902. (× 21).

Fig. 17b. Transverse section of a pair of ovules collected on the same date as that drawn in Fig. 17a. (× 21).

Fig. 18. Longitudinal section (tangential to the pair of ovules) of an ovule collected March 19th, 1902. (× 21).

Fig. 19. Transverse section of a pair of ovules collected April 8th, 1902. (× 21).

Fig. 20a. Transverse section of a single ovule with its bracts collected April 28th, 1902. (× 21).

Fig. 20b. Longitudinal section of a single ovule without its bracts, collected on the same day as that drawn in Fig. 20a. (× 21).

Fig. 21a. Fertile terminal bud which would develope into a leafy shoot in the current year, surrounded by four of last year's leaves. Collected May 8th, 1902. (Nat. size).

Fig. 21b. Pair of ovules with subtending leaf from the fertile bud drawn in Fig. 21a. (Nat. size).

Fig. 21c. Longitudinal section (tangential to the fertile shoot) of a pair of ovules such as that shewn in Fig. 21b. (× 21). Collected May 8th, 1902. The position of the growing point, which does not shew in the plane of this section, is indicated by a dotted line between the ovules.

Fig. 22a. Pair of ovules with subtending leaf, collected May 21st, 1902. (Nat. size).

Fig. 22b. Longitudinal section of a single ovule collected May 21st, 1902. (× 21).

Fig. 22c. Embryo-sac mother-cell from an ovule collected May 21st, 1902. (× 600).

Fig. 23. Longitudinal section of part of the nucellus of an ovule collected May 17th, 1904, shewing embryo-sac mother-cell and the surrounding cells drawn in outline only. (× 300).

Fig. 24a. Terminal fertile shoot collected June 9th, 1902. A pair of ovules is seen in the axil of one of the lower leaves (Nat. size). cf., the terminal bud in Fig. 21a. which shews the condition of this shoot as it was a month earlier.

Fig. 24b. Embryo-sac mother-cell with nucleus in synapsis from the longitudinal section of an ovule cellected June 9th, 1902 (× 600).

Fig. 25. Embryo-sac mother-cell with nucleus in synapsis from a longitudinal section of an ovule collected May, 17th, 1904. (× 600). Note the brush of fibrils in the protoplasm at the basal end.

Fig. 26. Embryo-sac mother-cell with nuclear thread in the beaded condition. (× 600). From a longitudinal section af an ovule collected June 1st, 1904.

Fig. 27a. Ovule with its bracts removed. (× about 4). Collected June 24th, 1902. The arillus surrounds the base of the conical integument which is truncated by the micropylar opening.

Fig. 27b Embryo-sac mother-cell shewing part of the spindle of the reduction division ; from a longitudinal section of an ovule collected on June 24th, 1902. (× 600).

Fig. 28. Embryo-sac mother-cell shewing part of the spindle of the reduction division from an ovule collected June 24th, 1902. The preparation has been treated with aqueous iodine. This has caused swelling of the wall and chromosomes and has darkened the masses of starch grains which occupy the ends of the mother-cell at this stage. (× 600).

Fig. 29. Embryo-sac mother-cell after the completion of the reduction division ; from the longitudinal section of an ovule collected June 1st, 1904. (× 600).

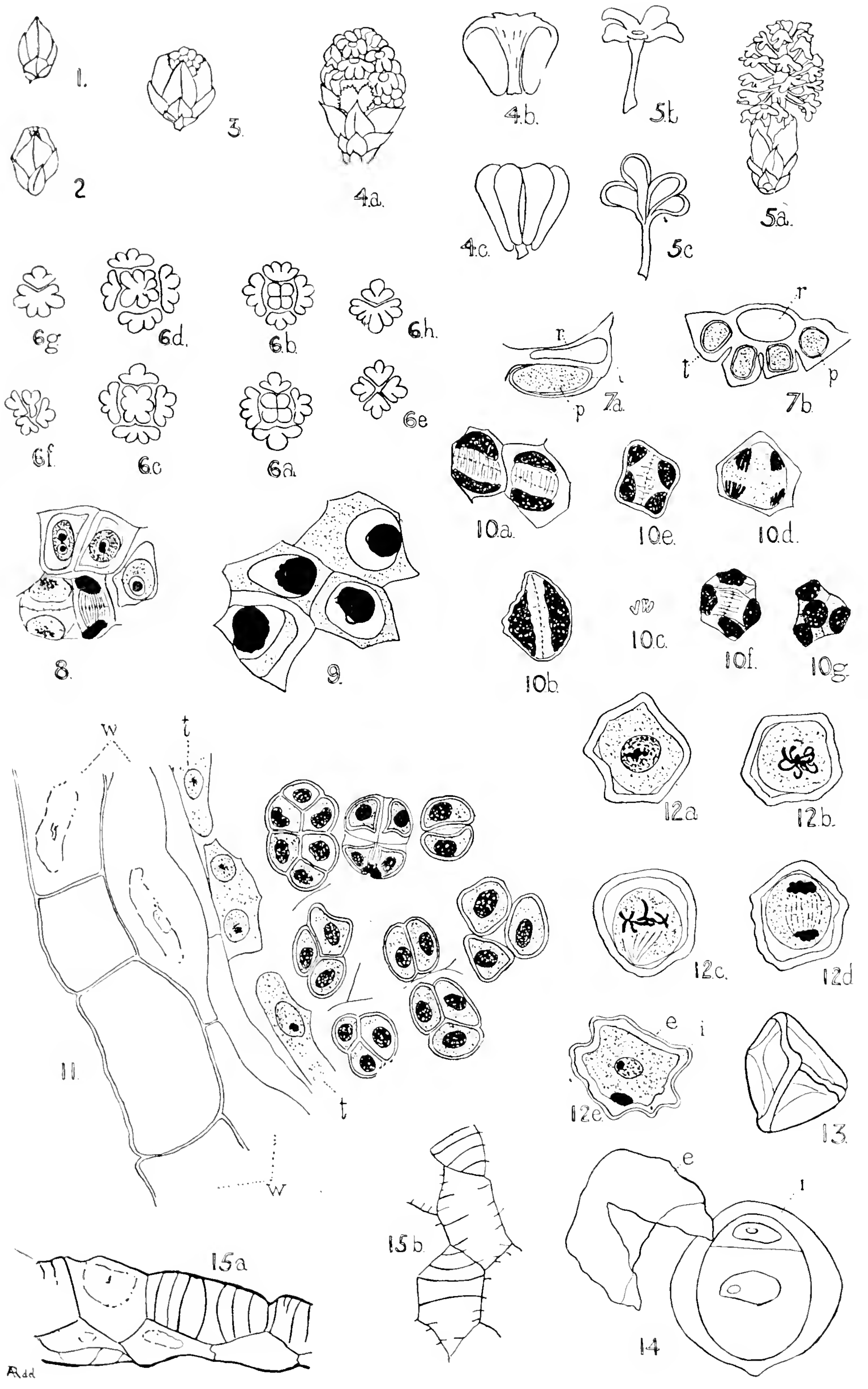
Fig. 30. Embryo-sac mother-cell from the longitudinal section of an ovule collected June 7th, 1904. The lower cell has already divided into two, while a spindle is visible in the upper cell. (× 600).

Fig. 31. Embryo-sac mother-cell from a longitudinal section of an ovule collected June 1st, 1904. The division into four spores has now taken place. (× 600).

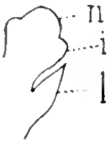
Fig. 32. Embryo-sac (megaspore) capped by three degenerating and flattened sister-cells from a longitudinal section of an ovule collected June 22nd, 1904. (× 600).

[Note.—All the microscopic figures have been drawn from single microtome sections, except Fig. 31, which was drawn from two successive sections.

The embryo-sac mother-cells are all drawn in the position which they would occupy if the axis of the ovule were vertical, and the micropyle was directed towards the top of the page.]



ROBERTSON—SPORE FORMATION IN *TORREYA.*



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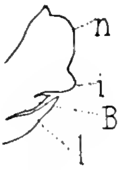

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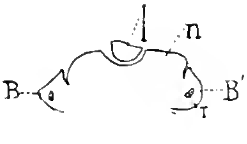

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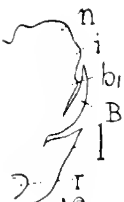

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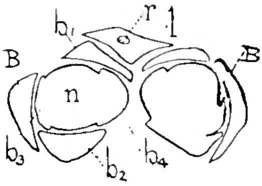

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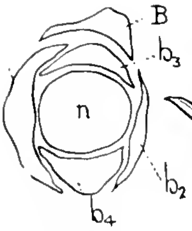

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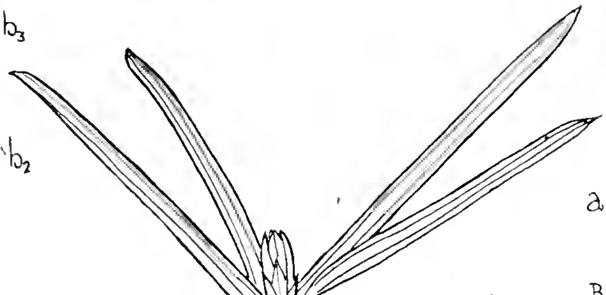
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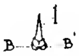

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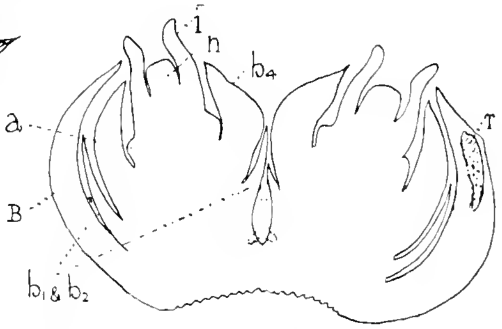

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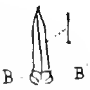

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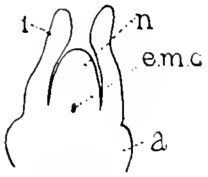

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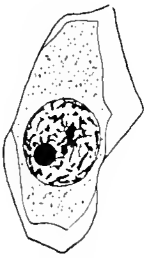
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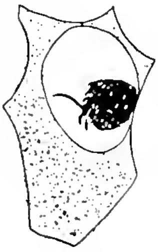
24a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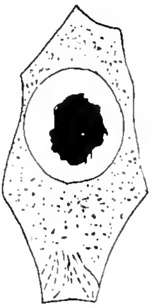
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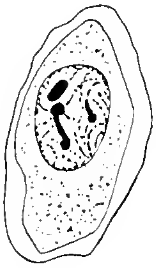
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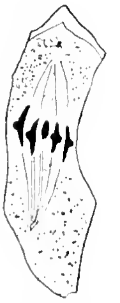
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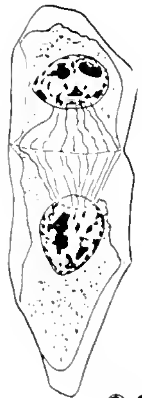
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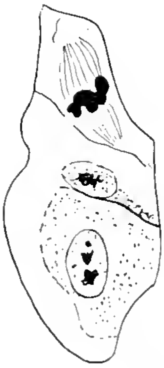
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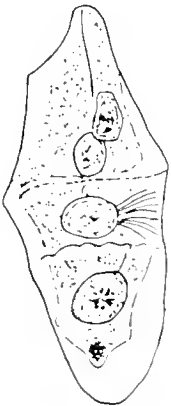
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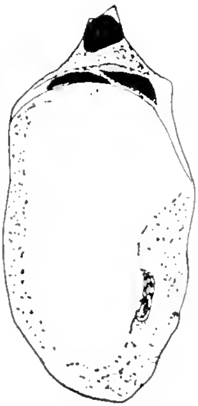
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ROBERTSON—SPORE FORMATION IN *TORREYA.*

ON THE RELATION OF FERTILISATION, "APOGAMY," AND "PARTHENOGENESIS."

BY VERNON H. BLACKMAN.

ALTHOUGH the subject of fertilisation (used in its widest sense to include all processes involving the fusion or association of gametes, *i.e.*, the *syngamy* of Hartog[1]) has been closely investigated for several decades, yet we are still unable to state the exact nature of the process. It is clear, however, that in typical exogamous fertilisation with a fusion of gametes from different individuals three obvious *main* results are brought about—a stimulus to further development, a mingling of two lines of descent, and a doubling of the number of chromosomes (or their equivalents).

That one of the important results of gametic fusion is a stimulus to further development has been recognised for some time and has led to the "rejuvenescence" theory of fertilisation. In this view the essential reason for fertilisation is to give new life and energy to protoplasm exhausted by continuous division ; it is a view based on the general facts of fertilisation and the usual inability of the sexual cells to develop without fusion. It received very striking support from the well known observations of Maupas who found that the descendants of single *Paramoecium* after about the 170th generation began to show signs of disorganisation, lost the power of division and soon died. When in this "senile" condition they could, however, be restored to their original vigour and power of division by *conjugation* with another individual (though not of the same brood).

These observations pointed clearly to the fact that conjugation (fertilisation) brought about some stimulus which restored the flagging energies of the organism. The probable nature of the stimulus has been made very evident by the brilliant observations of Calkins,[2] who found that, if, when the regular period of depression occurred in *Paramoecium* growing in hay-infusion, the organism were placed temporarily in beef, brain or pancreatic extract or even in a solution of potassium phosphate, the vigour of the cell was completely restored and when returned to the hay-infusion divided with its usual rapidity. By this means Calkins has been able to tide *Paramoecium* over several eras of depression, which would otherwise

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[1] Quart. Jour. Microsc. Science, XLVII. (1904), p. 581.

[2] Archiv f. Entwicklungsmech, XV. (1902), p. 139. Archiv f. Protistenkunde I. (1902), p. 355. Biol. Bull. III. (1902), p. 192.

have ended either in conjugation or death, and in one case to carry the organism beyond the 620th generation.

The replacement of conjugation by what must be a definite chemical stimulus is certainly a very striking observation and strongly suggests that during the temporary union the two individuals supply one another with chemical substances of which a lack has arisen in the organisms. The observed advantage of exogamous over endogamous conjugation would then be explained, as individuals of different broods grown under different conditions would be likely to have different needs, and by a process of interchange be able to supply each the other's wants. Calkins is of opinion that conjugation is much less common in nature than under artificial conditions, for every rainfall would bring into a pond in which *Paramoecium* might be living a change of dissolved substances and so obviate the necessity for conjugation.

These observations are in agreement with the well known observations of Loeb and others, in which normal eggs were made to develop for a time in a truly (but artificial) parthenogenetic way, by placing them in certain solutions, by alterations in temperature etc., or by placing them in a filtered extract of spermatic fluid (Winkler). There can thus be little doubt that one of the results of fertilisation is a chemical effect which brings about a change in the metabolism of the cells which unite.

Although in the majority of living organisms the process of fertilisation occurs sooner or later in the series of cell-divisions, yet the need for such a process cannot be considered as an inherent property of protoplasm, for such organisms as the Bacteria, Cyanophyceæ, and Mycetozoa seem to be completely *agamic*. Either their protoplasm is of such a nature that it does not become exhausted by a prolonged series of divisions or else a rejuvenescence is brought about in some other way, either by long periods of rest or by change of environment. In a few species also of the higher animals, males have never been observed and the only method of reproduction seems to be that of so-called parthenogenesis; also in some few Angiosperms, and perhaps in *Chara crinita*, the life-cycle is carried on without any form of gametic union (syngamy), although sexual organs are present.

That the process of union of gametes from different individuals brings about a mingling of different ancestral characters is, of course, sufficiently clear, but whether this process or that of protoplasmic rejuvenescence is to be considered as the essential, primary purpose of fertilisation is still very doubtful. Weismann has attacked the "rejuvenescence" theory of fertilisation and considers that the primary purpose of the process is to bring about variations; the need of fertilisation to renew the waning vigour of the protoplasm he considers to be secondary in nature and to have been later acquired. Darwin, Spencer, Oscar Hertwig, Strasburger and others believe on the other hand that the chief importance of fertilisation is to prevent too great variation and to keep the species true to the normal.

If the view, first put forward in an unsatisfactory form by Cannon, and later in an acceptable form by Sutton,[1]—that it is the reduction-division which leads to a segregation of maternal and paternal characters in accordance with Mendel's law,—be correct, it is clear that that division and the ensuing fertilisation would be a potent cause of variation; for of the gametes arising from an individual whose parents differed in a considerable number of characters hardly any two would be alike, and these would fuse with gametes equally dissimilar.

There can be little doubt, however, that, as other workers have stated, exogamous fertilisation tends not only to produce small variations, but also to keep them within narrow limits by a constant process of mixing; thus both views may be accepted. But it must be pointed out that the generally accepted view that individuals formed asexually are exactly like their parents has lately been shown to be quite erroneous. Warren[2] in his studies on the "parthenogenetic" eggs of *Aphis*, and Simpson[3] in *Paramoecium* have demonstrated that individuals produced asexually from a single parent may differ very considerably among themselves.

There can be little doubt, then, that the process of exogamous fertilisation has the effect of renewing in the gametes (partly it seems by a direct chemical effect) the full power of cell-division if that power has been lost or decreased; of mingling two lines of descent and so bringing about and controlling variations; and of doubling the number of chromosomes (or their equivalents) in the cell. These effects would seem to have been produced in what was probably the primitive form of fertilisation, that of the fusion of free-swimming

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[1] The Chromosomes in Heredity, Biol. Bull. IV. (1903), p. 231.

[2] Proc. Roy. Soc., LXV. (1900), p. 155.

[3] Biometrika I. (1902), p. 400. Weismann also earlier recognized variations in parthenogenetically developed species of *Cypris*.

unicellular organisms, for the two conjugating individuals would very likely be far separated in descent and would probably have spent their lives under slightly different conditions.

Although these, apparently primitive, results of fertilisation are still retained in the typical process, as observed in the higher organisms, yet there are to be found in animals and plants a series of *reduced* processes in which one or more of these characteristic results of fertilisation have been lost. The first step in such a process of reduction would seem to be the union of gametes more or less closely related in descent, so that fertilisation no longer brings about the mingling of different hereditary properties. There are many examples in plants, from "self-fertilisation" in Angiosperms to the union of gametes of the same brood among the Thallophyta.

"Self-fertilisation" among Angiosperms is no doubt a reduced process for the primitive forms would seem to have been cross-pollinated by the wind. It is, of course, not a process of true self-fertilisation, for it is not a fusion of gametes derived from the same individual, but merely that of gametes derived from distinct gametophytes, which, however, take their origin from, and are enclosed within, the same sporophyte. A process of true self-fertilisation is, of course, impossible in plants which are *heterosporous*; it does, however, appear sometimes to occur in homosporous forms such as *Osmunda* and *Ceratopteris*,[1] and seems to be found in such animals as the Trematoda.

A large number of cases of close in-breeding are to be found in the *Phycomycetes* amongst the fungi, where the sexual organs often arise on neighbouring cells or hyphae *(e.g., Peronosporaceae, Basidiobolus)*; in *Sphaerotheca* and *Pyronema* among the Ascomycetes the sexual organs would also seem to arise on neighbouring hyphae on the same mycelium. In these cases there can be no mingling of ancestral characters and they are certainly to be looked upon as physiologically simpler types of fertilisation when compared with the more complex types in which there *is* a mingling of such characters. There is little doubt also that they are to be considered as reduced in evolution, when one considers the fact that these fungal forms are probably derived from algal ancestors in which the male elements were either motile or free-floating, and so had opportunities of bringing about cross-fertilisation. The loss of freedom of the male cells in a land plant must almost of necessity lead to

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[1] R. P. Gregory. Proc. Camb. Phil. Soc. XII. (1904), p. 433.

in-breeding, unless there are special means, as in the Spermaphyta, of bringing the two plants into relation[1].

The relationship between the nuclei which unite in *Basidiobolus* is very close; according to the latest researches of Woycicki[2] the nuclei of neighbouring cells unite after they have undergone two divisions. In the aecidium of *Phragmidium*,[3] however (and probably also in other forms) a further stage of reduction is to be observed, for there the female cell, which is differentiated and has a sterile cell above, is fertilised by the entrance of the *nucleus* of a neighbouring, undifferentiated cell which passes through the wall, the two nuclei which become associated in the female cell being sometimes of such close relation that they separated in descent merely by a single division (loc. cit., figs. 68 and 69). That this process is reduced in evolution is clearly shown by the presence of abortive spermatia which have all the characters of male cells.

A still further stage of reduction of the process of fertilisation is to be seen in the case of "apogamy" in Ferns investigated by Farmer, Moore and Digby,[4] who found the nucleus of one vegetative prothallial cell migrating into another cell and fusing with the nucleus of the latter, thus bringing about the doubling of the number of chromosomes. The term *apogamy* was invented by De Bary to apply to all cases in which fertilisation appeared to be absent and would include cases of parthenogenesis; it has of late years been confined to those cases of transition from the gametophyte to the sporophyte without an obvious sexual process. It is evident how unsatisfactory is such a term when applied to a case which it is necessary to considered as the union of closely related and undifferentiated gametes, since it can be linked on to normal fertilization through such a process as that observed in the aecidium, where the female cell is differentiated, though the acting male cell is undifferentiated. It would seem best to speak of both these processes as *reduced forms of fertilisation*, for they are clearly reduced both physiologically (in the close relationship of the fusing nuclei) and in descent.

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[1] This does not seem to be always the case, for Blakeslee (Science XIX. (1904), p. 864, has lately made the interesting announcement that in some species of *Mucor* ("heterothallic" forms) there are two "strains" of mycelium, and that *zygospores* are only produced when hyphae from mycelia of the two "strains" come in contact. There is thus a differentiation of the nature of sex; and the well-known difficulty of producing zygospores in some forms is explained.

[2] Flora, XCIII. (1904), p. 87.

[3] V. H. Blackman. Annals of Botany, XVIII. (1904), p. 323.

[4] Proc. Roy. Soc., LXXI. (1903), p. 453.

A consideration of the cases of so-called parthenogenesis shows still further stages of reduction. A number of cases of thi process have been described amongst animals and plants but the majority of them, as Hartog[1] has pointed out, can lay no real claim to true parthenogenesis, which is the development of a *potential* gamete without relation to any other gamete. It is well-known from the observations of Weismann and Ischikawa, Blochmann, Henking, etc., that parthenogenesis occurs in *Rotifers*, in various groups of Crustacea (*Daphnidae, Polyphemidae, Artemia*) and in various insects (*Aphides, Hymenoptera*), but in the majority of cases the cell from which the embryo develops is not a normal egg, but one which has not formed a second polar-cell and is really the mother-cell of two gametes[2]. It has not undergone a reducing divisions and thus has the nuclear characters of a somatic cell with the double number of chromosomes, and is incapable of fertilisation. Clearly the formation of actual gametes has in these cases been suppressed and the process of development partakes of the nature of *somatic budding*.

A stage of development which connects most strikingly this process of somatic budding ("parthenogenesis") with that of normal fertilisation is that described by Brauer for the eggs of *Artemia salina*. Though the majority of the "parthenogenetic" eggs of this animal form only one polar-cell, yet a second one is produced in a few cases. This, however, is never extruded from the egg, but its nucleus, after moving a short distance away, returns to the egg nucleus and behaving like the nucleus of a spermatozoon fuses with the latter and thus brings the chromosomes up to the normal somatic number again.[3] The normal process of fertilisation is thus replaced by an endogamous union of sister gametes.

A certain number of cases of this form of "parthenogenesis" have been observed in Angiosperms, and allowing for a difference due to the alternation of generation in the plants, the similarity with the process described above for animals is exact. The development of

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[1] Quart. Jour. Microsc. Science XXXIV., (1901) p.

[2] It is now well established that the polar-cells represent merely three abortive eggs, and that they, with the egg, correspond with the tetrad of spermatozoa which are formed from one mother-cell; the first polar-cell represents two abortive eggs, the division, however, being usually, but not always, suppressed.

[3] The failure, later, of Petrunkewitsch (Anat. Anzeig. XXI., (1902) p. 256) to observe this process is probably to be explained as Castle (Bull. Mus. Comp. Zool. Harvard XL. (1903) p. 204) suggests, by the fact that the former investigated only winter eggs.

an embryo from an apparently normal egg-cell has been observed in *Antennaria alpina*, in several species of *Alchemilla*, in *Thalictrum purpurascens* and *Taraxacum officinale*. It appears, however, that in *all* these forms the number of chromosomes is not reduced in the megaspore-mother-cell, and Juel has observed in *Antennaria* and later in *Taraxacum*[1] that there is no tetrad division but the embryo-sac mother-cell only divides once; Overton[2] has also observed in *Thalictrum* the absence of the second division. In these cases of "parthenogenesis" and in those of animals we have thus the special reproductive cells (*gonotokonts* of Lotsy),[3] which usually form a tetrad of cells and undergo a reducing division, dividing only once and showing no reduction of chromosomes.

That the term parthenogenesis is as unsuitable in these cases as in those of animals is clear when it is considered that the "gametophyte" and the "egg" have the double number of chromosomes from the beginning, so that in the absence of reduction fertilisation is quite unnecessary. The gametophyte here provides an interesting case of a tissue which it is difficult to place in the category either of gametophyte or sporophyte for it has the general morphological features of the one and the cytological characters of the other.

It is very evident that in these peculiar cases amongst animals and plants we have reached the lowest stage of reduction in connection with fertilisation. The mingling of different characters, the stimulus to development, and the doubling of the number of chromosomes have all disappeared; and from the standpoint of cytology the process is reduced to one of mere *somatic or sporophytic budding*. That it is a stage in the reduction of fertilisation is shown by the fact that the process still continues along the lines of sexual reproduction, and involves the formation of "gametophyte" and "egg." Also, through such a case as the union of sister gametes in *Artemia*, the special types of fertilisation to be observed in the aecidium of *Phragmidium*, and the types of fertilisation to be observed in many fungi, these processes of "budding" may be linked on to normal exogamous fertilisation. In fact the cell which develops the embryo may be looked upon as the result of a theoretical fertilisation, *i.e.* as the product of the union of the sister-cells which should have been formed (and are formed in *Artemia*) from the cell which

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[1] Arsk. for Bot. II. (1904) No. 4.

[2] Ber. d. Deutsch. Bot. Ges. XXII. (1904), p. 274.

[3] Flora XCIII (1904), p. 65.

directly gives origin to the parthenogenetic egg in animals, and the parthenogenetic gametophyte in plants.

The occurrence of true parthenogenesis of facultative gametes is well-known among the lower plants,[1] but in the case of the higher forms, in which the gamete has a reduced number of chromosomes, no case is definitely known of the development of a mature individual in this way. Either the organism is unable to develop with the reduced number of chromosomes, or the nucleus is unable to double its chromosomes by a process of splitting without dividing; or else a nuclear mechanism produced by splitting of the original chromosomes is not sufficient for complete development. The last hypothesis is probably the correct one, as the peculiar behaviour of the unfertilised egg of the honey-bee suggests.

The development without fertilisation of eertain animal eggs which have formed *two* polar bodies has been described (*e.g.*: in Rotifers, Daphnidae, some Insects), and these would appear to be cases of true parthenogenesis, but, with the exception of the eggs of the honey-bee (*Apis mellifica*), the exact cytological details seem never to have been worked out and there seems some doubt as to whether a reduction division takes place in all these cases, or whether the second polar body may not fuse again with the egg as in *Artemia*; or it may be that a process similar to that described for the bee takes place.

The only form in which the cytological details are throughly known is, as mentioned above, that of the honey-bee. Blochmann showed in 1889 that in this insect all the eggs form two polar bodies, the fertilized ones producing female and the unfertilised (parthenogenetic) ones producing males (drones). Petrunkewitsch[2] has investigated the development of the eggs very carefully, and states that in the case of the drone-egg a process of extraordinary interest takes place. The main body of the animal is formed from the egg itself, which doubles its number of chromosomes probably by a simple process of arrested division. The typical number (three) of

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[1] Our knowledge of the nuclear changes which takes place in connection with fertilisation in the lower plants (except for such groups as Fucaceae, Dictyotaceae and Uredineae) and in the Protozoa is very meagre. Except for the groups mentioned it would appear that in the majority of cases the gametes have nuclei which are somatic in character; the parthenogenetic development of such facultative gametes is therefore not surprising. The peculiar divisions before the fusion of nuclei to be found in such cases as *Actinosphaerium*, *Actinophrys*, *Spirogyra* and *Basidiobolus* would suggest that nuclear reduction may sometimes take place before karyogamy.

[2] Zool. Jahrb., Abt. f. Ontog. XIV. (1901) p. 573 and XVII. (1903) p. 481.

polar-cells is formed by the division of the first one into two[1] and, the three cells are not extruded but merely lie in the periphery of the egg. The second polar-cell then fuses with one of the products of division of the first and thus produces a nucleus with the normal, somatic number of chromosomes. This cell then undergoes a number of divisions and migrating into the body of the developing embryo *forms there the genital gland.* If these observations be correct, and there seems little reason to doubt there accuracy, the body of the drone is clearly of *composite* nature, the ordinary somatic cells being truly parthenogenetic in origin, while the genital gland is derived from an endogamous fusion of gametes of the relationship of cousins.

Observation such as these in *Apis;* the fusion of the sister nuclei in *Artemia;* the special methods to prevent reduction to be observed in animals and plants in the peculiar cases (usually termed parthenogenesis) in which a special cell is produced which develops like an egg-cell without fertilisation; the absence of evidence in plants, and the doubtful evidence in animals,[1] of the capacity of normal eggs with the reduced number of chromosomes to produce complete mature individuals in a parthenogenetic way; all suggest the importance to the organism of starting its life history with a complete (unreduced) nuclear mechanism. Though the chromosomes appear to be doubled again in the drone-egg, yet this does not seem to be sufficient for complete development, for the sexual cells are derived in another way.[2]

Such fusions of nuclei as are to be observed in the egg of the honey-bee and in that of *Artemia* cannot be conceived as either bringing about mingling of different characters or producing any stimulus to further development; they must be considered as types of reduced fertilisation which have for their purpose the mere provision of a complete nuclear mechanism, *i.e.* the doubling of the number of chromosomes. It seems then permissible to doubt whether such reduced forms of fertilisation as are to be observed in the

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[1] The doubtful nature of true parthenogenesis in animals is also recognized by Phillips (A Review of Parthenogenesis. Contrib. Zool. Lab. Univ. Pennsyl. X. (1903), p. 275.

[2] Delage states that even in artificial parthenogenesis and in *merogony* (the development of portions of egg cytoplasm which have been fertilised by spermatozoa) the full number of chromosomes may be restored in some vegetative way. There seems some doubt about this point and these results are certainly contrary to those obtained by Morgan on merogony, and by Wilson on the artificial parthenogenesis of the eggs of *Toxopneustes.* In no case, has a mature individual been obtained in this way.

gametophyte of an "apogamous" fern or in the aecidium of *Phragmidium* have any other function than that of providing a suitable nuclear mechanism; they also would then belong to the category of reduced fertilisations in which two out of the three obvious results of the normal process have been lost. It would seem that since a normal egg-cell is apparently unable to develop into a complete organism if it starts with the reduced number of chromosomes—and since only a few animals and plants have evolved a method of development in which chromosome reduction is done away with—a process of nuclear fusion is still necessary in the life history of a number of organisms, although they appear able to dispense with the other results which are brought about by a typical exogamous fertilisation.

The Fungi are very probably a group in which the metabolic need of "rejuvenescence" by fertilisation has ceased, or almost ceased, to be felt, probably, as Marshall Ward[1] has suggested, owing to their parasitism, heteroecism and general mode of life. In the lower forms the sexual cells are often of close blood relationship, and though in the higher forms more recent work has established the existence of nuclear associations and fusions, yet in all these cases (except perhaps the lower Ascomycetes) these processes are of such a reduced type[2] that they would seem to have little other function than that of doubling the number of chromosomes (or their equivalents)

A study of these various types of fertilisation shows how almost impossible of definition is that process, for not only does it appear that the fusion or association of cells or nuclei (*syngamy*) may have very different effects in different organisms, but it has been shown that the sharply characterised process of exogamous fertilisation with differentiated sexual elements may be connected by a series of processes of increasing degradation and simplification, with one which is nothing more than a peculiar form of somatic or sporophytic budding.

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[1] Quart. Jour. Micros. Science XXIV. (1884), p. 262.

[2] In Uredineae, an association of closely related nuclei in the aecidium or also in the mycelium, with a later fusion in the teleutospore; in Basidiomycetes, an association of nuclei, probably of neighbouring vegetative cells, with a later fusion in the basidium; in the higher Ascomycetes, a fusion of closely related nuclei in the ascus. The lower Ascomycetes, however, are excessively puzzling, for in them there are apparently two successive fusions, one in the oogonium and one in the ascus.

THE PRESENT POSITION OF CELL-WALL RESEARCH.

IN the following note a brief outline is given of some of the principal directions along which research into the constitution of the cell-wall has moved during the last few years.

Until quite recently the chemistry of the cell-wall was believed to be comparatively simple. It was thought that the cellulose wall, which is first laid down, could either remain unchanged or it might subsequently become altered by lignification, cuticularisation or by mucilaginous changes. We now recognise that this account is wholly inadequate. It has been shown by various writers, notably by E. Schulze, that the cellulose of the older botanists is not a single body but a group of chemically allied substances. Schulze limits the term *cellulose* to that particular form which yields grape-sugar or dextrose on hydrolysis; a number of other similar bodies which do not split off dextrose but some other simple sugar (mannose, galactose, etc.) when they are hydrolysed are classed together as *hemi-celluloses.* Up to the present these hemi-celluloses have been almost entirely studied in connection with the seeds of various plants, especially (but not exclusively), in the thickened walls of the endosperm or cotyledon. Later research will no doubt show them to have a much wider distribution. The "*reserve-cellulose*" found by Reiss in the endosperm of *Phœnix dactylifera, Phytelephas,* etc., is a member of the hemi-cellusoses. The *amyloid* studied by Nadelmann, Reiss and Winterstein in various seeds is probably an allied body but its true chemical position is still undecided.

The *pectic substances,* first made known to us by the work of Payen, Braconnot, Mulder and Fremy, have been found by Mangin to have a very wide distribution in the cell walls of plants. Mangin classifies these substances into (1) Neutral pectic bodies, *e.g.* pectose and pectine; (2) Acid pectic bodies, *e.g.* pectic acid. In the inner layers of the cell-wall of a great many tissues pectose is found to be intimately associated with celluloses. The middle lamella is usually composed of calcium pectate. Pectose is readily converted into pectine and it has been suggested that the calcium pectate of the middle lamella arises from the transformation of pectine under the influence of the widely distributed enzyme *pectase.*

In the opinion of many chemists pectic bodies are carbohydrates, and some have suggested their close relationship to hemi-celluloses. Mangin disputes these assertions and believes that the pectic compounds stand apart from the carbohydrates.

For many years a mucilaginous substance which Mangin has called *callose* was only known in its association with sieve-tubes. Mangin has shown that it has a much wider distribution. He finds it in the pollen-grains of various Conifers, Cyperaceae and Juncaceae, as well as in the pollen-tubes of *Plantago*, *Caltha*, *Narcissus*, etc., in the walls of the pollen-mother-cells, in the cystoliths of the Urticales, and in the calcareous hairs and pericarps of several Borraginaceae. I have found it in the spore-mother-cell walls of certain Hepaticae *e.g.* (*Anthoceros*). Callose is only known to us by its micro-chemical reactions. Its true chemical nature is as yet quite obscure. *Cuticularised and suberised walls* have been very thoroughly studied by Gilson and Van Wisselingh. Both these authors believe that cellulose is entirely absent in suberised walls. Various acids have been obtained from the cork of different plants. These are *phellonic acid*, *suberic acid* and *phloionic acid*. Van Wisselingh regards cork-material or *suberin* as a fatty body which consists of glycerine esters and other compound esters as well as some further substance or substances which do not melt, are insoluble in chloroform, but are decomposed by K.O.H. solutions. *Cuticularisation* he regards as allied to but not identical with *suberisation*. It is due to the deposition of *cutin*.

Phellonic acid is absent in cutin. The optical relations of cork indicate that its double refraction is due to the presence of regularly arranged particles of crystalline form which melt on heating and re-crystallise on cooling (Ambronn). Numerous attempts have been made to identify the substance or substances which cause the *lignification* of the membrane. Tiemann-Haarmann concluded that *coniferin* formed a constituent of lignified membranes. Singer believed that the "lignin-reactions" depend upon the presence of *vanillin* as well as that of coniferin. Nickel and Seliwanow found that an *aldehyde body* occurred in wood. Czapek has recently pointed out that all the reactions which were used for the identification of the various bodies mentioned above are untrustworthy. By boiling with zinc chloride Czapek has separated a substance which he names *hadromal* and which he believes is partly or entirely responsible for lignification. The concomitant of hadromal in the lignified wall is presumably cellulose and it is probable that the constituent of the lignified wall which produces the lignin-reactions is an hadromal-cellulose-ether together with a trace of free hadromal. Mäule has described a reaction for lignified tissue which can still be obtained after the abstraction of hadromal. He concludes from this that Czapek's hadromal cannot be the true lignin but is only a constant concomitant of this. It should be mentioned, however, that Czapek himself had suggested that more than one cellulose-ether may possibly occur in lignified membranes. The *membranes of Fungi* have been the subject of careful examination by Gilson, Winterstein and Van Wisselingh. *Cellulose* and *chitin* are the most important constituents. These two bodies do not occur together but each is usually accompanied by other, generally undetermined or little known substances (*e.g.* lichenin). Cellulose was only detected in (1) three Peronosporeae which were examined; (2) Saprolegnia; (3) A few Myxomycetes. A small quantity of cellulose may possibly exist in *Rhitisma salicinum* whilst in *Geaster* and *Usnea* bodies occur which react similarly to cellulose (*geastrin* and *usnein*). Chitin was only rarely found wanting in the membranes of the higher Fungi but the quantity in which it occurs varies greatly. The Zygomycetes, Chitridiaceae, Entomophthoreae and some Myxomycetes contain chitin. In Bacteria, *Saccharomyces*, *Fuligo septica* and *Cetraria islandica* neither chitin nor cellulose were found by V. Wisselingh. Callose does not usually occur in fungal membranes. Pectic bodies are occasionally found; Lagerheim found these, accompanied probably by chitin, in the walls of the Monoblepharideae.

An interesting contribution to the chemistry of the cell-wall in various Mosses is made by Czapek. He finds that the cellulose is seldom free in these membranes but is usually combined either with a phenol-like body—*sphagnol*—or with a tannin-like substance—*Dicranum-tannic acid.*

After this brief review of the chemistry of the cell-wall it remains for us to consider the manner of its formation by the protoplast and its subsequent growth and differentiation. It has been a matter of frequent discussion whether the wall is formed by a direct transformation of a layer or mass of cytoplasm (Pringsheim) or whether it is produced by the secretory activity of the protoplast (Von Mohl). A large number of cases are now well established in which the wall is unquestionably formed on the surface of the protoplast by secretion. In some other cases the whole cytoplasm appears to become "transformed" into cell-wall material. This has been described (Strasburger, Tischler, Buscalioni, Schmitz and

Magnus) in the trabeculae of *Caulerpa*, the embryo-sac of various Scrophulariaceae and Plantaginaceae, the massulae of *Azolla*, the epidermal cells of the seed coat of several plants, in the suspensor cell of *Phaseolus* and in the cells of the roots of several plants which form mycorrhiza. As Tischler points out we cannot correctly speak of a direct "transformation" of protoplasm, which consists largely of proteids, into the carbohydrate material of a cell-wall. No doubt the carbohydrate is split off from the proteid molecule whilst soluble nitrogenous substances remain over. In accordance with Pringsheim's theory it was long taught that the cell-plate, which is formed during cell-division, is directly converted into the primary cell-wall separating the daughter cells. This view has now been shown to be erroneous by the work of Treub, Strasburger and Timberlake.

The cell-plate splits to form the plasma membranes of the daughter-cells and the new cell-wall is secreted between them. The double nature of the cell-wall thus secreted by the two new plasma membranes is strongly supported by the observations of Allen on the middle lamella. There is accumulating evidence to show that each new wall and each fresh lamella which is added to a wall is only formed in the most intimate connection with the protoplast (Klebs, Strasburger). That not only the cytoplasm, but also the nucleus is concerned in this work is clearly shown by the work of Haberlandt, Klebs, Townsend, Derschau and Gerassimoff.

What relative share is taken by the nucleus and by the cytoplasm in the organisation of a new wall and in the manufacture of the material necessary for its formation cannot yet be decisively stated. In nearly all cases which have been observed the nucleus can be clearly seen to play a prominent part in both processes, whilst in one case at least I have observed that the constructive material for the growth of the cell-wall is manufactured at the expense of the trophoplasm, whilst kinoplasm and a somewhat shrunken nucleus remain at the conclusion of the process.

In his most recent contribution to cell-wall literature (1898) Strasburger has shown that growth in surface of a membrane takes place either by passive stretching, accompanied simultaneously by the deposition of new lamellae on the face of the stretched membranes, or by intussusception (Substanz einlagerung). Growth in thickness generally takes place by the deposition of new lamellae upon those already present. These lamellae do not usually grow in thickness by intussusception (Substanz einlagerung), but in certain cases especially in free cells (*e.g.* pollen-grains), such a growth in thickness of the lamellae, often accompanied by remarkable differentiation of form and structure, does take place. Mention should here be made of Schütt's work upon the *centrifugal growth* in thickness of the membranes of certain Peridineæ and Diatomeæ. This growth takes place by the agency of protoplasm which has flowed out from the interior of the cell through pores in the membrane and has reached the outer surface of the wall. One other question of importance remains to be considered before closing this note. It is that regarding the manner in which the differentiation of a cell-wall lamella is effected after it has once been formed by the protoplast, and, in some cases, become separated from the protoplast either by the interpolation of fresh lamellae or by the actual contraction of the protoplast itself. The remarkable observations of Fitting upon the macrospores of *Isoetes* and *Selaginella* have shown us a case in which the protoplast is entirely separated from a membrane which still continues to grow and to differentiate. Fitting's work has been confirmed by Denke and by Campbell. Moreover, I have some, as yet unpublished, observations upon the pollen-grains of certain Onagraceae in which I have found quite comparable phenomena. Both in sections of material fixed in various fluids and in living material examined in physiological salt or sugar solutions or in the plant's own juice the protoplast was seen, at certain stages, to be contracted away from the growing and differentiating membrane. If these observations are a true record of what occurs in the normal cell—and the most critical examination of the pollen-grains has hitherto shown nothing to oppose this view—it becomes an exceedingly difficult matter to offer an explanation of the manner in which the differentiation of such a membrane is effected.

It is possible that the explanation must be sought for in the way in which the original molecules are deposited in the lamella when this is first formed in intimate attachment to, and under the direct agency of the protoplast. These molecules may, not improbably, be arranged after a definite and specific plan and they may be regarded as exerting an influence over the positions in which all subsequent molecules are set, even after the directive agency of the protoplast has been removed. The process might be roughly compared to that of a complex crystallisation system in which the form and the position of the crystals is determined at the outset by the living element of the cell. Perhaps a deeper study of the finer structure of the lamellae, may help to throw some light on this difficult problem. It should also be recalled in this relation that Gilson obtained cellulose in a crystalline form, that Arthur Meyer has advocated the crystalline nature of starch-grains, and that Ambronn explained the optical properties of cork by a reference to the crystalline constitution of cuticularised and suberised membranes.

RUDOLF BEER.

INSECT-ATTRACTIONS IN FLOWERS.

IN an article entitled "What part does colour play in the attraction of insects to flowers" published by Mr. Tansley in the New Phytologist (Vol. III., No. 2, p. 51), the author discusses this question ápropos of the experiments of Plateau and Andreae. The papers of these naturalists I have not seen, but I think that perhaps a few notes of observations made in the tropics may be of some interest and perhaps throw light on the question.

"Andreae concludes," says Mr. Tansley, "that of the day-flying insects, the biologically higher types, *i.e.* those with a relatively long life in the winged state, and with a long direct flight, are characterised by sharp sight and are attracted to flowers mainly by their colour, while the biologically lower forms, *i.e.* those with a short life in the winged condition and a short flight, have less power of sight and are more dependent on smell" (p. 54). I should venture to traverse this as too general a statement. Insects are so varied in their habits, in the tropics at least, that those of the same group may be apparently attracted either by scent or colour according to circumstances, or rather according to the habits of the species. Thus for example the great blue-black butterfly, *Papilio Esperi* dashes about in the mid-day sun along jungle paths and only stops at red flowers most of which are quite scentless at that time, at least to an ordinary observer. If a piece of red paper or cloth, or a fallen Hibiscus flower lies in its track it will often stoop at it and inspect it, clearly showing that it is attracted by colour only. The other Papilios such as *P. Sarpedon* dash about in a similar way, but are chiefly attracted by the white-flowered Eugenias, the red or orange *Lantana* and other more distinctly scented plants.

Amathusia Phidippus, as well as the *Thaumantidae*, only leave the dark recesses of the jungle at dusk, when brightly coloured flowers are practically invisible, and consequently do not attract them, nor do they visit the white nocturnal flowers. Fallen fruit however proves a great attraction, and these butterflies often find their way into moth-traps baited with bananas. I have heard of as many as thirty Amathusias being taken in a single moth-trap.

The only time I have ever so far as I remember seen *Amathusia* at a flower was one early morning when I saw one drinking water from the flower-head of *Nicolaia imperialis*, but of course not fertilizing the flowers.

The *Sphingidae* are mostly nocturnal or rather crepuscular, and almost certainly go by scent in seeking honey, but doubtless the white colour of the flower serves to call their attention to the source of the odour. The flowers most visited by these insects are Crinums, *Lonicera*, *Asystasia coromandeliana* (primrose-coloured variety), *Isotoma*, and a *Hymenocallis*. These flowers, except *Asystasia*, are only scented at night, and during the day are only visited by *Trigona*, a bee always on the search for pollen and any sticky substance which it can utilize for its resinous nest. *Sphinx convolvuli* often comes into the house in the evening, when there is a bunch of flowers on the table and will go all round the bunch picking out the flowers of the honeysuckle, and probing their tubes with its proboseis, taking no notice of the other flowers.

Although there are many diurnal white flowers in the gardens, Eugenias, *Rhodamnia*, *Eucharis* and the like, I never saw any of the *Sphingidae* take any notice of them. These diurnal flowers are quite scentless by night.

The humming bird hawk-moths *Macroglossa* and *Cephonodes* seek for honey in the evening before sun-down, disappearing at dusk. They seldom visit the nocturnal flowers, but search for such plants as *Lantana*, *Duranta* (flowers blue), *Cleome speciosa* (pink), Coffee (white, and strongly scented in the afternoon), and other such plants. They like the other *Sphingidae* seem more attracted by scent than colour. But some years ago I observed a curious fact which seemed to me to show that these insects, at least, went very much by form, regardless of colour or scent, and I do not think that due attention has been paid to the attraction to insects of the form of flowers. While passing through the Suez Canal on a steamer a number of Macroglossas (apparently *M. Stellatarum*) flew into the smoking-room. This room had all round it a row of white enamelled tiles on which were rude representations of flowers in blue. The figures were alternately of a Chrysanthemum-like composite and a cup-shaped blossom more or less resembling a tulip. The moths flew round the room, passing over the chrysanthemum and visiting the tulip-like flowers, attempting to probe with their proboscides the conventionalised mouths of the flowers. Now there could be no doubt that here neither scent nor colour attracted the insects, but that the form, conventional as it was, of the painted flowers was what directed them in their pursuit of nectar.

In the case of Diptera again I feel that we must distinguish between the groups. The pollen eating *Syrphidae* frequent *Porana*, *Rhodamnia* and Eugenias, with white flowers, and certainly seem to go by colour, rather than scent. The *Muscidae* on the other hand seem to go exclusively by scent. The coarse-smelling green flowers of *Kurrimia paniculata*, *Castanopsis*, *Quercus* and *Sindora* are visited by myriads of a species of *Musca*, so that the roar of their wings can often be heard on a lofty *Sindora*, from the ground. The rapidity with which *Muscidae* discover a piece of carrion or other foul-smelling putrid matter even when covered up is I suppose well-known to all. In this connection the fertilization of the Amorphophalli is very striking. These plants, *A. Titanum*, *A. Prainianum* and *A. Rex*, when the spathe is fully expanded, all give out a carrion, like odour, perceptible for twenty yards or more, for about twelve to twenty-four hours or less, and are then attractive to *Muscidae*. After this the smell quite ceases and though the spadix lasts for another two days otherwise unaltered, no flies are to be seen on it. These Amorphophalli are all differently coloured, but there is always a dark maroon-purple patch at the base of the spathe inside. This colouration also occurs at the entrance to the tube of *Aristolochia gigas*, and *A. elegans*, and, I take it, may be classed as a large guiding mark for a class of insects whose power of vision is poor, and may be limited to distinguishing dark and light only. Another fly of a different group, but not identified, constantly visits the orchids *Bulbophyllum macranthum* (see Annals of Botany, Vol. IV., p. 327), *Phalaenopsis violacea* and *Dendrobium superbum*. I have never seen it on other flowers, and rarely on any other occasions, but when either of these plants is in bloom these flies appear in numbers. They fertilize the *Bulbophyllum* certainly, but I do not see that they fertilize the *Dendrobium*. They seem only to run about over the petal and sepals. The *Bulbophyllum* has a scent of cloves, and I attempted to attract the flies to pieces of paper scented with oil of cloves, but without success. However the scent of the oil is even to me different from that of the *Bulbophyllum*, and is probably more so to the flies. The *Dendrobium* smells strongly of rhubarb. I observed that though *Dendrobium phalaenopsis* was in flower close to the *D. superbum*, no flies left the latter for the former, although the scentless flowers of *D. phalaenopsis* have much the colouring and form of those of *D. superbum* ; and also that partly opened flowers of the latter before they emitted any scent, but after the colour could be seen, were not noticed by the flies. Rough coloured representatives of this flower in paper were equally overlooked.

The scents of these two orchids are quite different, but I have no doubt that the scent is the attraction and neither colour nor form in this case. Since writing the above I see that the flies have moved from the withered *Dendrobium superbum* to a flower of *Phalaenopsis violacea* which has a distinct though not very strong scent.

In the matter of attraction by scent it may well be that insects can detect it more readily than we can. Fallen fruits such as those of *Arenga saccharifera* and *Elaeocarpus ganitrus*, or Bananas, have to us little scent, yet they are very attractive to Lepidoptera, and some apparently almost scentless flowers seem to attract insects from considerable distances.

In powerfully scented flowers it appears that the widely diffused scent attracts any insects which come within its area, but that then they are guided to the flower by its colouring or form. The scent is strongest usually in that part of the flower where the nectar lies hid, but this is not always the case. The orchid *Renanthera moschifera* is an example. The strong musky odour is produced from the back of the recurved tip of the upper sepal. The rest of the flower is scentless. The sepals and petals are green, with brown blotches, but the lip is white, and quite conspicuous for some distance. The flower is usually fertilized by *Vespa cincta*, but neither this insect nor any other, have I ever seen to go for even a second to the scented sepal ; once attracted to the locality of the flowers even if fifty yards away, the insect seems to espy the white lip and fly straight to it.

In conclusion I would suggest that scent alone is rarely sufficient for the fertilizer, with the sole exception here at least of the *Muscidae*, who seem to be unable clearly to distinguish colour, further, than dark and light, but that the form of the flower plays a conspicuous part in the attraction and that while bright colour, red, white and yellow is serviceable to attract wide-ranging diurnal insects, white will not attract nocturnal insects unless accompanied by a powerful scent.

Singapore, May, 1904. H. N. RIDLEY.

[Mr. Ridley's very interesting observations on the whole confirm Andreae's main conclusions very strikingly.—Ed. New Phytologist.

FURTHER OBSERVATIONS ON THE POLLINATION OF THE PRIMROSE AND OF THE COWSLIP,

BY F. E. WEISS, D.Sc.

IN an article which appeared last year in this journal (Vol. II., Nos. 4 and 5) I stated as the result of a series of careful observations that the primroses, at all events in the part of Shropshire where my observations were made, are fairly regularly visited by a number of different insects in fine sunny weather. The fact that many observers have been unable to detect such insect visitors I attributed to their observations having been made either in cold or dull weather, or in exposed and windy situations. For even on sunny days I could not observe the usual insect visitors on primroses in wind-swept localities, while, at the same time, in sheltered positions some larger humble-bees might occasionally be met with on the primroses, and *Anthophora*, *Bombylius* and pollen-gathering Andrenae fairly regularly. These observations and the conclusions drawn from them with regard to the cross-pollination of the primrose were directly opposed to the views of the anonymous author of "The Primrose and Darwinism," the late Rev. Edward Bell, and in a a recent number of *Nature Notes* (April, 1904), appeared an article by him criticising my conclusions. It was with great regret that I read at the end of the article the announcement by the Editor of *Nature Notes* of the untimely death of the Rev. Edward Bell through the upsetting of an oil lamp.

Some of the criticisms referred to I have already answered (*Nature Notes*, June, 1904), but as my original article appeared in the NEW PHYTOLOGIST I should like to contribute to this journal both a correction and some additional observations.

Through an unfortunate disarrangement in the labels of a collection which I consulted for the purpose of identifying the insects observed on the primroses, I was led to give the wrong specific name to one of them. The Anthophora which I observed and collected was *Anthophora pilipes* and not *furcata*, which latter insect I am told does not appear until later in the season. Except for that correction I adhere to the views previously expressed that the primroses are not only adapted to the visits of insects but are actually cross-pollinated by at least some of the insects which visit them. That self-pollination also takes place I have, as previously stated, no doubt; but to effect this, some agency is necessary, such as the wind or some minute insects like thrips which are very commonly met with in the flowers in some localities. In the absence of these agencies these plants remain sterile, as I have clearly proved this spring with plants of both the short and long styled forms grown under glass. That the glass itself did not in any way interfere with the fertility of the plants, as Mr. Bell supposed the nets used by Darwin did, is shown by the fact that control-plants, self and cross-pollinated by artificial means matured their seeds readily. The fact of the matter is that the truth lies between the two extreme views, the one put forward by Mr. Bell who disbelieved in the natural cross-fertilisation of the Primulaceae and the other extreme maintained by Lord Avebury who has stated that heterostyly is "one of the principal modes by which self-fertilisation is prevented." ("Flowers and Insects.") A microscopic examination of the stigma of a large number of primrose flowers from very different localities has shown that a considerable portion have pollen-grains from both the long-styled and the short-styled flowers fixed among their hairs, and there is no difficulty in distinguishing the two kinds of pollen. The proportion of the large and small pollen-grains varied very much and as the flowers were not examined on the spot it is impossible to make any deductions from the relative number of the two kinds of grains. As a rule there were on the stigma more pollen-grains similar to those produced in the flower itself, but the presence of even a few pollen-grains of a larger or smaller size shows that the visits of the insects have been effective in producing cross-pollination, and if, as Darwin showed, this pollen possessed any prepotency, the number of cross fertilized ovules might be a quite considerable proportion of the total seeds produced.

With regard to the insects I had observed last year I was anxious to find out whether the same insects were the fertilising agents in other localities, especially as Mr. Bell in a private letter to me had expressed a doubt whether *Bombylius*, which I had found most frequently on the primroses at Church Stretton, was common enough to account for the cross-pollination of so widespread a flower as the primrose. I therefore visited another locality this spring. Unfortunately the Easter Vacation was rather too early this year to see the primroses at their best, and I had some difficulty in North Staffordshire, where I was staying, to find a sheltered bank where the primroses were plentiful and sufficiently forward. But on two mornings out of the three on which I visited a favourable spot I found the bee-fly (*Bombylius*) busy on the flowers of the primrose, and eagerly sucking the honey from them. It is interesting to note that on one of these days the two Bombylii I then observed were the only insects I noticed flying at all, the morning being somewhat cold. There were no bees or other insects on the celandines which grew in great profusion near the primroses. I have also received details of some very careful observations made this spring in Westmoreland by Miss Mary L. Armitt, an accomplished field naturalist, who was good enough to send me her notes.

On April 19th, a thoroughly summerlike day, Miss Armitt found the bee-fly *(Bombylius)* busy among the primroses near the foot of Nabscar, Rydal. In less than half-an-hour more than a dozen flowers were visited. Following up one insect it was seen to visit two flowers on one root both being thrum-eyed; then it went to another root and visited two flowers which were pin-eyed. Another bee-fly that was at work at the same time was also followed in its visits first to a short-styled and then to a long-styled plant. On a subsequent day Miss Armitt was successful in detecting a bee-fly visiting ten flowers in a quarter of an hour, one flower on one root, four on the next, three on another and two on a fourth plant. These records by so competent an observer should go far to confirm the observations made by me in two different localities and to mark out *Bombylius* as the chief agent in the cross-pollination of the primrose, and the result of the examination of the stigmas is clear evidence that these visits are not without effect.

I was in a position this year too, to make some observations on the cowslip, and on Whit Monday particularly I had an opportunity of watching for insects in two fields in which cowslips were very plentiful.

In the first meadow, which was very exposed, I saw no insects visiting the flowers, but in the second meadow, which was sheltered from the strong wind by a copse of trees, I observed five humble bees, each of which visited quite a large number of flowers. The insects proved to be specimens of *Bombus muscorum* and *Bombus terrestris* This quite confirms Darwin's statement that the "cowslip is habitually visited during the day by larger humble bees" a statement strongly controverted by Mr. Bell who states that he "failed to see a single instance of such a visit to the flowers by humble bees."

An examination of the stigmas of the cowslip both of the long styled and short styled forms reveals the presence of pollen-grains from flowers of a different form as well as pollen from the same or similar flowers, so that here too both cross and self-fertilisation takes place. This in fact we must conclude from observational evidence takes place in both the primrose and in the cowslip.

THE ROTHAMSTED AGRICULTURAL EXPERIMENTS.

THOUGH Rothamsted is a household word to agriculturalists all the world over, and the value of its long series of experiments are doubtless fully appreciated, it may be questioned if botanists in general are fully aware of the importance and extent of the purely botanical aspects of the work that is being carried on at the station for scientific experiments in agriculture founded more than sixty years ago by the late Sir (then Mr.) John Bennet Lawes on his estate close to the little town of Harpenden in Hertfordshire.

The history of the station is briefly as follows. In the year 1843 Mr. Lawes, who had succeeded to the Rothamsted property in 1834 and being of a chemical turn of mind had already experimented with various chemical manures and obtained excellent results with "superphosphates" for turnips, results which had led him to patent their manufacture in 1842, started the systematic series of experiments which have been continued down to the present day. He seems to have been partly spurred thereto by the lectures of Liebig delivered at the Royal Institution. Liebig taught that plants derived not only their carbon but also their nitrogen from the atmosphere, and on this he founded his doctrine of *mineral manuring*, or the putting back into the soil of the ash constituents which the plant has taken from it. Lawes found that this theory did not square with his practical experience, and selecting certain fields from his estate, and devoting a barn to the purposes of an agricultural laboratory, began the great series of experiments which have been carried on continuously from 1843 till the present day.

In that year also Lawes obtained the services of Dr. (afterwards Sir) Henry Gilbert to take charge of the laboratory, and Gilbert remained as Chief Chemist and Director till his death in 1901. So long ago as 1854, a number of leading agriculturalists, desiring to shew their appreciation of the work, presented Lawes with the present laboratory, and the work of several chemical and general assistants was from an early date involved in carrying out the routine work of analysing soil samples and ash constituents. In 1889 Sir John Lawes with far-sighted munificence set aside a trust fund of £100,000 as well as the 50 acres or so of experimental ground, and the laboratory and sample room, to be bequeathed to a trust for the continuance of the work after his death, which took place in 1900. This is not the place to give even the briefest summary of the splendid results of Lawes' and Gilbert's work. Our present object is to direct attention to some of the more striking botanical lessons to be learned from a visit to Rothamsted at the present day.

On the death of Sir Henry Gilbert at the end of 1901, Mr. A. D. Hall, at that time Principal of the South Eastern Agricultural College at Wye, was appointed Director of the Station by the Lawes Trustees, and he is not only carrying on the unbroken series of manuring experiments inaugurated by Lawes and Gilbert, but has also started important new lines of research.

Some of the lessons of the Rothamsted experiments were developed by Mr. Hall in the lectures he gave last autumn for the University of London at the Chelsea Physic Garden on "The Constitution of the Plant in Relation to Soil," and many of those who attended these lectures have recently had an opportunity of visiting Rothamsted and seeing for themselves, under Mr. Hall's guidance, some of the effects of the differences of nutrition to which various agricultural crops have been subjected for so many years.

EFFECT OF MANURING ON MEADOW-LAND.

Perhaps the most striking and instructive experiments from a general botanical point of view are those which shew the effects of long continued differential manuring of meadow land. In 1856 an area of about seven acres of land in Rothamsted Park, which had been permanent grass for at least 200 years, was divided into a number of plots for differential treatment, and since that time each plot has been continuously treated with a definite quantity of a particular manure. Twice every year the grass is cut and an estimate is made, not only of the total weight of hay from each plot, in cwts. per acre, but also of the botanical composition of the hay, *i.e.* of the percentages of the different species of plants in the total dry weight of the crop.

The cumulative effect of the differential manuring, not only on the quantities of hay produced but especially on the species of plants growing on the different plots, is most stirikngly evident to an observer visiting the experimental area in the middle of June, just before the cutting of the grass. One plot will be bright with buttercups and sorrel, while the adjoining one shews not a single flower, but is entirely composed of tall dark-green meadow-grasses, and a third is covered with the graceful white umbels of Anthriscus.

This striking effect, it must be remembered, has been brought about in what was originally perfectly uniform meadow-land, entirely by differences of manuring, which have so changed the conditions of the struggle for existence in each individual plot, as to bring about the success of a different combination of species in each case, and thus to produce a number of distinct floras living side by side, bounded by mathematically straight lines and apparently never trespassing across them.

Of course the component species of these floras are all inhabitants of meadowland of one kind or another, and several are common to the majority of the plots, but it is a striking fact that some of the plants which occur on plots subjected to the most extreme conditions, particularly on the starved plots, are not only not found on the ordinary grass land adjacent, but are nowhere common in the surrounding country side. Thus for instance *Poterium Sanguisorba*, the Salad Burnet, characteristic of dry chalk downs is found only and is very common on the two most starved plots, *i.e.* the plot which has been unmanured since 1856, and the plot which has been exhausted by treatment with superphosphate of lime alone since 1859; while *Briza media*, the Quaking Grass, universally taken as a sign of poor land, occurs in any quantity only on these two and on the plot which has no potash. Neither of these plants are conspicuous, at any rate, in the countryside. It would be an enquiry of some interest to determine what are actually the nearest habitats of these species, from which presumably the colonisation of the two plots must have occurred.

The two most starved plots bear a varied herbage (47 species) consisting mainly of weede (*i.e.* plants which are of little use as constituents of hay, and which stock generally refuse) and are very poor in grasses, particularly in the valuable ones.

Manuring with combined nitrogen, on the other hand, either in the form of ammonium salts or nitrate of soda, though in the absence of the other mineral manures it gives but a poor and weedy crop, encourages the grasses at the expense of the leguminous plants.

A complete manure, consisting of the necessary mineral constituents together with combined nitrogen, enables the grasses largely to suppress the competition of the "weeds" and completely to exclude the leguminous plants. With increase in the amount of combined nitrogen the "weeds" become still fewer, and the weight of hay rapidly increases, though the number of *species* of grass becomes very restricted. Thus in one very heavily "over-manured" plot there are only ten species of plants altogether, and 99·8% of the dry weight of the crop is composed of grasses, almost entirely of three species. This crop usually goes flat before cutting, and is particuarly liable to the attacks of fungi.

Treatment with a mixed mineral manure, including the indispensable potash, but excluding nitrogen, has caused a great increase of leguminous plants (mainly clover), which sometimes form 50% of the dry weight of the crop. This is of course because these plants, being independent of combined nitrogen, when they are supplied with the indispensable potassium, gain a great advantage over the grasses, which do require combined nitrogen. There are, however, actually more grasses in the hay of this plot than in that of an unmanured plot, because these plants live at the expense of the combined nitrogen in the humus formed by the leguminous plants.

The specialisation of the vegetation, according to whether the food-constituents in the manures are retained at the surface or penetrate more deeply into the soil, is also very clearly brought out. Thus the plots manured with ammonium salts bear a shallow-rooted vegetation with such grasses as *Festuca ovina* and *Anthoxanthum odoratum*, while those manured with sodium nitrate give deep-rooted crops with *Bromus mollis* and *Avena elatior* dominant. The plot manured with a complete manure, the nitrogen as sodium nitrate, is also strikingly characterised by the abundance of *Anthriscus sylvestris*, which, as is well-known, ordinarily frequents hedge sides and the shade of trees, often in company with *Bromus*. Why the nitrate manuring should encourage it in the open is not at all obvious.

We have only alluded to a few of the more important conclusions that may be deduced from this extremely beautiful series of experiments. They suggest subjects for fresh enquiry on every hand. Perhaps the most important general lesson is the emphasis given to the keen competition of different species in the struggle for existence, and the enormous advantage given to certain species over others by comparatively slight alterations in the conditions of life. The working of some of these alterations we are able to trace, while others still remain a mystery; but there can be no doubt that long continued systematic experiment of this kind is the surest means of throwing light on the often perplexing combinations of species in natural floras.

WHEAT.

Broadbalk Field, about eleven acres in extent, has been continuously under wheat for sixty-one years, parallel strips having been treated with different manures.

Even the unmanured plot still continues to produce a crop every year, and its average crop for the last half century (thirteen bushels to the acre) is about the same as the world's average, though the English farmer expects a much heavier crop than this. The plot receiving a mixed mineral manure without nitrogen, produces very little more than the unmanured plot; with nitrogen the crop increases greatly, and a progressive increase of nitrogen leads to a corresponding increase in the crop. Thus we see, as we saw in the case of hay, that combined nitrogen is *the* great requirement of the grass-plant. One of the great objects of changing the crop on a piece of land is to get rid of the weeds especially associated with a given crop, which would otherwise increase to a harmful extent. This is seen on Broadbalk field, where the wheat had become so "foul" with *Alopecurus agrestis*, the Black Bent Grass, that the experiment is being tried of fallowing half of each plot in alternate years, in order to get rid of the pest.

One end of Broadbalk field was left entirely to itself many years ago. In comparatively few years the wheat entirely disappeared, its place being taken by a miscellaneous mixture of weeds, among which were a number of seedling bushes and trees. In another few years the bushes formed an impenetrable thicket, through which the young trees, largely oaks, were beginning to push their way. In another thirty years there would have been a young oak wood with rich undergrowth, but the bushes were in the way and have been recently stubbed up. The succession of events illustrates the constant tendency of good land to go back to the condition of woodland (no doubt its primitive state throughout the temperate regions) a tendency only arrested by the constant cutting of crops or by grazing.

CLOVER-SICKNESS.

A very interesting experiment with leguminous plants has been made in Hoos Field.

Various leguminous crops have been grown continuously since 1848 with various combinations of mineral manures, but after the first few years they became a complete failure, owing to an unknown cause which nearly always prevents the continuous growing of leguminous crops and is called by farmers "clover sickness." The bulk of this area was ploughed up in 1898 and sowed with wheat. Five crops of this were taken without manuring in succeeding years to test the amount of nitrogen accumulated by the leguminous plants and left in the soil. Heavy crops of wheat were taken the first year, but in subsequent years they fell off greatly, in some cases to the "unmanured" level at once, in others more gradually. The residue of nitrogen left by lucerne is still palpable six years after the discontinuance of its growth. This year oats have been sown instead, and in the oats, leguminous plants again. This will shew if the ground has recovered from the "clover-sickness." Meanwhile the extreme ends of the original "clover-sick" strips are still under their impoverished leguminous crops, and wretched plants, for the most part, they are.

Many interesting lines of research that are being carried on at Rothamsted cannot be entered into here. No mention for instance has been made of the manuring experiments with root crops, nor of the investigations which Mr. Hall is pursuing with the object of trying to understand that still mysterious property, the "strength" of wheat, or rather of the dough made from it, a property which enables certain foreign wheats, such as American and Russian, to fetch substantially more in the market than the best English-grown crops. But enough has been written to give some idea of the extraordinary botanical interest attaching to the work which was begun by Lawes, and which his admirable generosity has made it possible to continue indefinitely. A. G. T.

AN EXHIBIT OF SPECIMENS OF SEED-BEARING PLANTS FROM THE PALÆOZOIC ROCKS.

THE readers of this Journal have been kept informed as to the recent additions to our knowledge of the early fern-like Gymnosperms.[1] On the occasion of the recent conversazione held by the University of London on the 27th of May, a considerable collection of specimens was brought together by the chief workers in this field, illustrating not merely the newer work, but as far as possible also the habit and appearance of the palæozoic plants known to bear seeds or seed-like structures.[2]

In view of the fact that a new group, the Pteridospermeae

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[1] New Phytologist, Vol. II., p. 73; Vol. III., pp. 32 and 102.

[2] The following sent contributions :—Mr. E. A. Newell Arber, Miss M. Benson, Mr. R. Kidston, F.R.S., Professor F. W. Oliver and Dr. D. H. Scott, F.R.S.

seems to be emerging from the series of the Cycadofilices, till last year known only by their vegetative organs, the suggestion has been made that it would be of some interest if an authentic record of the specimens exhibited could be drawn up, more especially as many of them are quite unique and it is hardly likely that they will ever be assembled together again.

The present note is an enumeration of the specimens shewn, which fall under four headings. (1) The *Cordaiteae*, a group of Palæozoic gymnosperms long known to fossil-botanists, (2) the *Lyginodendreae* with their "Lagenostoma"-seeds, (3) the *Medulloseae*, and (4) *Lycopodineae* which include Lepidocarpon and Miadesmia, both with seed-like fructifications. Groups (2) and (3) constitute, for the moment, the proposed new class, Pteridospermeae.

It may be added that the whole collection has been accepted for temporary exhibition by the Trustees of the British Museum, and that they will remain on view at the Natural History Museum (where they have been admirably arranged by Mr. E. A. N. Arber) for some little time.

In the following enumeration the numbers of the specimens in their respective collections is given, when available, in square brackets thus [ ]. K = Kidston Collection, S = Scott Collection, U.C.L. = University College, London, Botanical Department Collection.

In its present form the Collection is enhanced by the inclusion of additional specimens belonging to the Geological Department of the Natural History Museum. For the purposes of this note the specimens (or groups of specimens) are also numbered consecutively.

(I.)—CORDAITEAE.

Foliage :

1. *Cordaites (Dorycordaites)*, entire leaf; M. Coal Meas., Barnsley, [K 2907].
2. *C. principalis*, Germar, apex and base of leaf; M. Coal Meas., Barnsley, Figured, Trans. Roy. Soc., Edin., vol. 37, pl. iv., figs. 8 and 16. [K 1478 and 9].
3. Phots. vasc. bundles of leaves, *lingulatus* and *principalis* types. Permo.-carb., Grand 'Croix. Figured, New Phyt., vol. II., pl. ix., figs 1 and 3 [U.C.L.]
4. Leafy branch, *Dammara robusta*, for comparison.

Stem :

5. *Cordaicladus* (branch of *C. principalis* with leaf-scars) ; L. Coal Meas., Kilmarnock. Figured, Trans. Roy. Soc., Edin., 37, pl, iv., fig 17 [K 1561].

6. Polished slab, large Cordaitean stem, Lennel Braes.

7. Phot. large stem, 27-ft. high, in situ, Lennel Braes.

8. Large trans. sectn. [Binney, G1].

9. Series of radial longitl. sectns. with discoid pith [U.C.L., Binney 214, Binney, G, 4].

10. "*Sternbergia approximata*," Newcastle [U.C.L.]

11. *Pitys antiqua*, Witham, trans. sectn. and phots. of stem. Figured. Trans. Roy. Soc., Edin, vol. 40, pt. 2., pl. I., phot. 5, pl. v., fig. 15. Lennel Braes, Calc. Sandstone Series, [K598A.]

Reproductive Organs.

12. *Cordaianthus Pitcairneae*, the female inflorescence, M. Coal Meas., Barnsley [K 2374].

13. *Cardiocarpus annulatus* and *C. Gutbaeri*, seed impressions.

14. *Taxospermum*, section of petrified seed from Grand 'Croix; wax model of seed (after Brongniart).

15. Drawing of restoration of *Cordaites (Doryeordaites)*, whole tree, and branch of *C. laevis*, both modified from Grand 'Eury.

16. *Gingko biloba*, phots. and living specimens, including young seeds from the well-known tree at Montpellier. Sketch of tree by a Chinese artist, from Seward and Gowan, Ann. of Bot., vol. XIV., pl. 8.

(II.)—LYGINODENDREÆ.

Lyginodendron Oldhamium, Will.

17. Restoration of plant, original drawing by J. Allen, reproduced as frontispiece in Scott's "Studies," 1900.

Foliage.

18. Impressions of *Sphenopteris Hoeninghausi*, Brongn., showing the frond, also indications of "spines" on rachis and pinnules, and of the "Dictyoxylon" cortex of the former [K 937, 940, 941, 2935.]

19. Sections showing petrified foliage [S 1161, 1162.]

20. Sections and drawings of leaf-trace of *Lyginodendron* and of the petiole of a recent Cycad shewing their close anatomical agreement. Cf. Scott's "Studies," p. 316.

Stem.

21. Cast of possible stem of *Lyginodendron* showing the elongated meshes on the surface [U.C.L.]

22. Tangential section of a petrified stem with "Dictyoxylon"-cortex for comparison.

23. Large transverse section of stem and drawings [S 648.]

Reproductive Organs.

24. *Calymmatotheca Stangeri*, Stur. Drawings of the type-specimens in the Geological Museum, Vienna. The cup-like structures terminating the branching rachis were perhaps of the nature of involucres enclosing seeds or pollen-sacs.

25. *Telangium Scotti*, Benson. Drawing of sporangial clusters supposed to be the pollen-bearing organs of a *Lyginodendron*.

26. *Lagenostoma Lomaxi*, Will. Enlarged phot. of a small seed enclosed in its cupule—from a negative by L. A. Boodle. [Will. Coll., 1931.A]

27. *L. Lomaxi*. Longitudinal section of a full-sized seed [U.C.L. R1.]

28. Large scale model of the seed and another of its apex with removable parts by H. E. H. Smedley.

29. Large restoration in wax of a small-sized seed in its cupule.

30. Photographs from specimens illustrating the identity in structure of the cupular glands of the seed with glands attached to the rachis and stem of *Lyginodendron Oldhamium* (from Scott & Oliver, Phil. Trans. B., Vol. 197).

31. *Lagenostoma ovoides*, Will. Petrified specimen complete, *in situ* [K.]

32. Phots. of various sections of this seed.

33. *Lagenostoma physoides*, Will. Large model and phots. of sections of the seed, including phots. of three transverse sections of one seed from the collection of the late M. Hovelacque.

34. *Lagenostoma Sinclari*, Kidston, M.S. Three impressions shewing attachment of supposed seeds to branching rachis. [K. 3529, 3530, 3531.]

35. *Lagenostoma* sp., large slab covered with impressions of ribbed seeds resembling *L. physoides*. [Glasgow University Museum.]

(III.)—MEDULLOSEÆ.

Foliage.

36. *Neuropteris heterophylla*, Brongn. Portion of frond, L. Coal Meas., Whiterigg, Lanark [K 3526.]

37. The same, detached pinna, L. Coal Meas., Barnsley [U.C.L.]

38. The same, impression of rachis with two Cyclopteroid pinnules attached [K 97.]

39. Detached Cyclopteroid pinnule [K 3527.]

40. Drawing of unusually perfect frond showing relation of the Cyclopteroid pinnules [after Roehl and Zeiller.]

41. *Alethopteris lonchitica*, Brongn. Impressions of fronds from M. Coal Meas., Barnsley, and sections of petrified pinnules from Grand 'Croix, for comparison [U.C.L.]

Stems.

42. *Medullosa anglica*, Scott. Petrified block showing surface of stem with leaf-bases [figured, Scott "Studies," p. 377.]

43. The same, trans. section of stem with phot. and diagram [S 737.]

44. Several slabs and sections of *Medullosa stellata* from Chemnitz [U.C.L.]

Fructifications.

45. *Neuropteris heterophylla*, Brongn. Drawing of supposed male fructification [after Kidston, Phil. Trans. B., Vol. 197, p. 3.]

46. The unique set of seed-impressions attached to recognisable portions of the leaf of *Neuropteris heterophylla*, from the Middle Coal Meas., South Staffordshire Coalfield, Coseley, Nr. Dudley, and including all the specimens figured by Kidston in Phil. Trans. B., Vol. 197, pl. I.

Trigonocarpus.

Seeds suspected of having been borne by a *Medullosa.*

47. *T. Parkinsoni*, Bgt. A series of impressions of specimens showing the stone enclosed in the fleshy layer of the testa; others showing cast of interior of seed and of the long micropyle. [K 591, 1579, 1062, 1063, 1064.]

48. Phot. of group of casts from a specimen in the York Museum.

49. Impression of several seeds showing showing association with pinnules of *Alethopteris lonchitica.*

50. *T. olivaeforme*, Will., trans. and long. sectns. of petrified specimens [S 325, 940], with a cast of interior of *T. Parkinsoni* for comparison.

(IV.)—LYCOPODINEAE.

Lepidocarpon Lomaxi, Scott, was fully illustrated by a selection of sections and drawings from the Scott Collection, including:—

51. Tangential sections of seeds [S 621, 861.]

52. Seed showing prothallus [S 1073.]

53. Cone in tangential section, seeds without integument [S 1848.]

54. Cone in transverse section [S 1866.]

55. Section of a very large seed belonging to an undescribed species of *Lepidocarpon* [S.]

56. *Lepidostrobus Brownii*, Schimper, trans. and longitudinal sections [Brit. Mus. Coll.]

57. Models in wax by Mr. Smedley of a seed on its bract as well as of an ordinary *Lepidostrobus.*

Sections and impressions of the vegetative organs of *Lepidodendron* were associated with these seed-like structures, including:—

58. *Lepidodendron (Lepidophloios) Harcourtii*, Witham, trans. sectn. of stem [Brit. Mus. Coll.]

59. The same, branching specimen, with several seeds of *Lepidocarpon Lomaxi* associated [U.C.L.]

60. Habit impressions of stems and leafy branches of various species of *Lepidodendron.*

61. Restoration of *Lepidodendron*, after Grand 'Eury.

62. *Miadesmia membranacea*, Bertrand, was on account of its smallness illustrated by photographs (from original specimens in the Royal Holloway College and University College Collections), diagrams, and more especially by an admirable series of models in plasticine.

F.W.O.

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THE CAMBRIDGE MEETING OF THE BRITISH 'ASSOCIATION.

THE meetings of the Association that are held in the old University towns must always possess a certain distinction and charm which separate them from those that take place in business centres. In the case of Cambridge, also, the vivid scientific life of the University, and its admirable equipment for the pursuit of the various branches of science, add enormously to the pleasure and profit to be gained from such a meeting of scientific men. In no branch of science can this be more marked than in Botany. The Cambridge school is *facile princeps* in this country, while its magnificent, and at the same time thoroughly workmanlike Institute, an account of the opening of which will be found in this journal for March last, offers an ideal location for the meetings of Section K.

With one or two exceptions every British botanist of note was present at the meeting, and the younger men, too, came in great force. There was also a record attendance of foreign guests, no less than twenty botanists from abroad, including many leaders of the science, coming to enjoy Cambridge hospitality.

The President of the Section, Mr. Francis Darwin, than whom there could be no more appropriate occupant of the Chair, delivered his address on Thursday morning. His subject was "The Perception of the Force of Gravity by Plants," and the address was devoted to a detailed consideration of the evidence for and against the two current theories of the mechanism of "gravi-perception"—the radial pressure theory and the statolith theory.

Though many of the phenomena involved are still obscure, and the whole subject is of considerable difficulty, Mr. Darwin concludes in favour of "the general though not the universal applicability of the statolith theory." The radial pressure mechanism suggested by Pfeffer and Czapek, may obtain, he thinks, in the Algæ and Fungi, where statoliths have never been observed. There is no *a priori* reason why the mechanism of gravi-perception should not vary in different groups of plants.

The rest of the morning was occupied by Dr. Scott's account of an interesting new type of Sphenophyllaceous cone, and by Professor Bertrand's account, illustrated by an immense series of lantern slides, of his and Professor Cornaille's most recent and very beautiful researches on the leaf-traces of Ferns. British fern-anatomists were extremely glad to find Professor Bertrand among them, and his paper promises to clear up some of the most difficult problems in the morphology of the leaf-traces of the Filicinean phylum.

In the afternoon Miss Stopes gave a too brief account of her recent thorough work on the morphology of Cycad ovules, and morphological papers by Mr. Arber, Mr. Boodle and Dr. Lang occupied the rest of the sitting.

Friday morning was devoted to Ecology. Mr. Tansley gave an introductory address on the Problems of Ecology, which will be found in the present number of this journal. Professor Engler welcomed the present tendency to devote attention to ecological work on the ground that it helped to prevent systematic botany from sinking into mere specialism. He called attention to the too frequent neglect of the cryptogams, which so often prepare the soil for the higher plants. Dr. Stapf was particularly glad that ecological work was being taken up in the British Isles, since their flora is of special interest on account of their geographical position and geological history.

Dr. W. G. Smith followed with an account of the progress of the botanical survey of Britain. After some introductory remarks on adaptation and plant-associations, he compared Flahault's method of survey with Drude's, and with that adopted by himself and his co-workers in the north of England and in Scotland. He pointed out that the tallest plants dominate an association, and hence naturally form the "index species." While trees play this part over great tracts of the earth's surface, this is not now the case (though at one time it probably was) in most of the native flora of Britain, and hence Flahault's tree-index method of mapping is artificial for most of our native associations.

Mr. T. W. Woodhead gave a most interesting paper on the "Biology and Distribution of Woodland Plants." The author has worked out the associations and some of the factors determining the distribution of the "character-plants" in the most excellent way, and his points were illustrated by many admirable photographs.

Professor Engler read an interesting paper on the migration of S. European plants to the High Mountains of Tropical Africa.

In the afternoon Professor Klebs read an important paper on the "Artificial Formation of a New Race." The author had succeeded in inducing artificially certain "mutations" in *Veronica Chamædrys*, for instance by excising the terminal bud, and these he found reproduced not only in cuttings, but in plants raised from seed and grown under ordinary conditions.

There was also a discussion between Mr. Wager and Professor Zacharias on the nature of the Cyanophyceous cell, and an address by Dr. Lotsy, illustrated by beautiful lantern slides, on the Vegetation in the Virgin Woods of Java.

Saturday was devoted to an extremely pleasant excursion to Chippenham Fen, in which nearly all the members of the Section took part, though it is to be feared that very few did any botanising! From a social point of view the excursion was most enjoyable. The thanks of all who took part in it are due to Mr. A. W. Hill, who looked after the arrangements, not least because the programme was so planned that no one was either hurried or bored the whole day.

On Monday morning after a paper by Lord Avebury, Professor Marshall Ward gave an address on "Recent Reserches on Parasitic Fungi," in which he dealt historically with the progress of knowledge on the relation of host and parasite, tracing it down to the most recent problems. Professor Eriksson followed with a contribution to the subject from the point of view of the "mycoplasm theory." This was followed by other papers on Fungi, which we have no space to notice.

On Tuesday morning Dr. F. F. Blackman gave an account, illustrated by apparatus, of the recent researches, by himself and Miss Matthaei, on "Sunshine and CO_2-Assimilation." The Section was delighted, as much by the extremely thorough and fundamental nature of the work, as by the extraordinary ingenuity and patience with which each technical difficulty was faced and surmounted as it arose. The President, Professor Reinke and Dr. Gardiner vied with each other in their tributes to the value of the authors' work.

Professor Vines followed with a lucid account of the most recent advances in our knowledge of the Proteases of Plants; Dr. Blakeslee gave an account of his striking and beautiful work on the remarkable sexual behaviour of conjugating hyphæ in the Mucorini; and Professor Errera and Dr. Lotsy read papers on alkaloids.

Mrs. Scott shewed her attractive Kammatograms in the small lecture room during the morning.

In the afternoon, Dr. Scott dealt with the recent discoveries of seeds among carboniferous plants under the title of "A new Aspect of the Carboniferous Flora."

On Wednesday morning Professor Fujii of Tokio communicated his recent discoveries on the Pollination of Gymnosperms. Professor Chodat read an important paper on "Oxidising Enzymes and Katalases in Plants," Dr. Miyake presented his results "On the Centrosome of the Hepaticæ," and some other papers were read, including a number on Fungi which had been crowded out of an earlier programme and were taken in the small lecture-room simultaneously with the main meeting.

This very incomplete and fragmentary account of the meetings of the Section will at least serve to convey an impression of the enormous number of papers read, as well as of their great interest. Many more were crowded out altogether. The great draw-back of the meeting was in fact the attempt to squeeze into a strictly limited time quite twice as many papers as could be adequately dealt with. The result is inevitable injustice, weariness and confusion, as well as the almost total elimination of useful discussion. The difficulty only arises in its acute form in the case of a large meeting like this one. The last bad case of congestion was at Glasgow in 1901. However, the problem of organisation ought not to be quite beyond the powers of the officers of the Section. It is successfully solved in America, and no doubt it can be in England. Probably the experience gained at Cambridge will be turned to good account at the York meeting in 1906.

There was an extremely successful dinner of the Section in the beautiful combination-room of St. John's College on Monday night, at which fifty-nine botanists were present.

Cambridge hospitality was true to its reputation. Most of the foreign guests were entertained at Sidney Sussex College, the Secretaries of Sections at Emmanuel. The arrangements were everywhere excellent.

NOTES ON INFECTION EXPERIMENTS WITH VARIOUS UREDINEÆ.

By Miss C. M. Gibson.

[With Plates V. and VI.]

THE infection experiments, the results of which are recorded in the first part of this paper, originated in a suggestion made by Professor Marshall Ward that I should see what happened if any plant, say *Ranunculus Ficaria*, were inoculated with the uredospores of Chrysanthemum-rust.

Ranunculus Ficaria was chosen as host from the convenience of the surface of its leaves for inoculation and from the fact that it had large stomata. Uredospores of Chrysanthemum-rust were chosen to inoculate with, because a large stock of this rust happened to be available at the time.

The following list summarises the results of successful and doubtful cases of the entry of the stomata by the germ-tubes of the spores employed:—

Kind of Spore used.	Species of Fungus.	Original Host.	Experimental Host.	Result.
Uredospores	*Uredo Chrysanthemi*	*Chrysanthemum sinense*	*R. Ficaria*	enter freely
Aecidiospores	*Phragmidium rosae-alpinae*	*Rosa*	,,	,, ,,
Aecidiospores	*Uromyces Poae*	*Ranunculus repens*	,,	,, ,,
Aecidiospores	*Aecidium Bunii*	*Bunium*	,,	negative
Aecidiospores	*Puccinia Poarum*	*Tussilago*	*Caltha*	enter freely
Uredospores	*Uromyces Geranii*	*Geranium*	,,	,, ,,
Aecidiospores	*Puccinia Menthae*	*Mentha*	,,	enter not very freely
Uredospores	*Puccinia*	*Carduus*	,,	enter freely
Aecidiospores	*Phragmidium Sanguisorbae*	*Poterium*		2 or 3 doubtful entries
Uredospores	*Puccinia glumarum*	*Triticum vulgare*	,,	1 certain entry
Uredospores	*Puccinia graminis*	*Poa aspera*	,,	enter freely
,,	*Uromyces Poae*	*Poa pratensis*	,,	,, ,,
,,	*Puccinia Taraxaci*	*Taraxacum*	*Tropæolum*	,, ,,
,,	*Coleosporium Sonchi*	*Tussilago*	,,	,, ,,
,,	,, ,,	,,	*Valeriana*	,, ,,
,,	*Puccinia pulverulenta*	*Epilobium*	,,	1 entry
,,	*Puccinia Centaureae*	*Centaurea nigra*	,,	enter freely
,,	*Puccinia Menthae*	*Mentha*	,,	enter freely

The results obtained were interesting enough to suggest that the same experiment should be tried with other rusts. Therefore as the season advanced and the various rusts appeared, as many as possible were obtained, and inoculations made with the uredospores, or aecidiospores as the case might be. Quite a number of the spores refused to germinate for reasons not apparent, so that no observation could be made on their infecting powers. The results obtained from those which did germinate are given on page 184. Towards the end of May *Caltha palustris* was substituted for *R. Ficaria*, as the latter plant was dying down for the summer. At the beginning of August it became impossible to keep Caltha alive, so Tropæolum and Valeriana were used instead.

In all these cases the germ-tube enters the stoma as in a normal infection on its own host plant. The after course of events is however quite different. The stage of development to which the entering tube attains before dying varies, but death ultimately results in all cases. In some few cases the germ-tube never gets right through the stoma, but forms a swelling in the actual slit and there stops (Fig. 1). In other cases it forms a substomatal swelling and emits from this one or more hyphae (sometimes as many as six, Fig. 2) just as in normal infection. These hyphae may grow to a greater or less length before dying, from a mere projection to three-fourths the depth of the spongy parenchyma (Figs. 3, 4, 5, 6, 7 and 8). A very common case is that in which the germ-tube forms, in the stomatal slit, a swelling which projects into the substomatal space. This swelling may develope no farther (Figs. 9 and 10) or from the side of it a hypha may grow till it meets with a cell and at the point of contact form a swelling and so ends its growth (Figs. 11, 12, 13 and 27). Another very common case is for the germ-tube to form no swelling at all, either in or under the stoma, but to grow straight on till it meets a cell (which may be at a considerable depth in the leaf) and there form a swelling (Figs. 14, 15 and 16) or else swell along its whole length and so come to an end (Figs. 17 and 18), though in rare cases the swelling emits a hypha (Fig. 19).

From this it will be seen that in the struggle against adverse circumstances the entering germ-tube tries all kinds of plans quite different from its behaviour in normal infection.

The length of time that these hyphae remain capable of growth varies; but in no leaves four days after inoculation can hyphae be found which have not obviously reached the end of their capacity for growth. In some cases in two-day preparations, hyphae are found already dead and shrivelled, and this is very frequently the case on the third day. But however long they keep up the battle, or however vigorous they appear at first, the hyphae invariably lose in the struggle for existence. In all the preparations of these infections that I have examined I have never found a single haustorium.

The facts seem to suggest that the death of the entering hyphae is not due so much to starvation as to some poisonous substance emitted by the cells; for on examining an epidermis freshly stripped from a leaf inoculated three days before, the hyphae outside are seen to be alive and unshrunken while a great number of those inside are already dead and shrivelled. Also in microtome sections of the same date after inoculation the swellings and hyphae inside the leaf were often shrivelled and empty, while the hyphae outside were still full of protoplasm (Fig 20). Sowings of uredospores from Chrysanthemum were also made in water to compare with those made on the leaf and after three days fixed and stained; it was difficult to keep the culture any longer than this as it became so over-run with Penicillium. In the case of those examined the protoplasm had disappeared from most of the hypha, being confined to a short distance behind the tip; in most cases however it had not degenerated, and had preserved its original structure, although the nuclei, when seen at all, often shewed signs of degeneration. There was certainly not as much shrivelling as is often seen in the hyphae inside the plant. The whole question is however difficult to decide, as the hyphae seem to vary in their powers of resistance, dying and vigorous ones being found in the same infection spot (Figs. 21 and 22). The leaves also seem to vary in their power to kill, one set of sections containing only dead and shrivelled hyphae and the next set of the same age containing mostly living ones.

Although the observations recorded above were for the most part made on stained microtome sections of hardened material, parallel observations were made on the living leaves by stripping off the epidermis and examining its under surface. In all cases in which the result is given as positive the germ-tubes have been seen projecting through the stomata and forming beneath them swellings shewing the characteristic colour of the protoplasm of the particular spore used for infection in that case. All substomatal swellings not shewing the characteristic colour were ignored.

From the above results it is evident that infection is a much more complicated matter than the mere entry of the stoma by the germ-tube. In the first place it is clear that entrance of the stoma by any germ-tube is no index of the capacity of that germ-tube to infect the leaf. If we may generalise from the cases given above, the germ-tubes of the spores of any member of the Uredineæ may enter any leaf and grow there to some extent, while it is well-known that the capacity for infection of the spores of any one species is strictly limited.

What causes the entry of the germ-tubes is at present unknown to us; probably as Miyoshi and others have supposed, it is the attraction of some substance to which the germ-tube is positively chemotropic, but far from its being a special substance for each plant, the evidence suggests that it is some substance common to all plants. We do not even know if it is liquid or gaseous; it seems possible it may be the latter, the stomata and inter-cellular spaces being emphatically an apparatus for gaseous inter-change.

The second point to notice is that, it is the power of the hyphae to form haustoria, which we must take as an index of infective capacity; because, if the fungus cannot use the host-plant as food, it must shortly die of starvation. Whether the incapacity to penetrate the cells is due to lack of attractive substance or to the presence of anything actively repellent is not clear, though as before stated, certain facts seem to suggest the presence of something harmful to the hyphae.

I will now proceed to certain results obtained in the course of a series of experiments on Chrysanthemum-rust.

This rust exists the whole year through in the uredo-stage. Certain observers have reported the discovery of teleutospores in the months of December and January. It is impossible to contradict this statement, because the rust on individual plants may vary in this respect, but the occurrence of teleutospores is not general, for out of multitudes of spores, gathered from a collection of some hundreds of plants on many occasions from October to March, I have never found any teleutospores, nor have I found any in the pustules on my own plants which I have examined from time to time for the purpose.

And indeed there is no necessity for resting spores, for the young shoots are above ground long before the old ones die away, and these young shoots are taken as cuttings, so that there is no time when there are no leaves upon which the rust can live.

The time of year when there is an apparent break in the life of the fungus is from May to September. In fact so impressed was I by the disappearance of the rust in summer and its re-appearance in autumn that I hunted the stems of the plants for a perennial mycelium, but found none. I then went and examined a collection which had been very badly attacked by the rust in the previous autumn, but which the gardener said was now quite clean.

I, too, thought this to be the case for some time till one small pustule was discovered. There may of course have been one or two more, but even that one would be quite enough to account for an extensive outbreak when the plants were crowded together in a greenhouse.

The histology of the fungus in its normal state was gone into, but shewed little of special interest, except the fact that the haustoria often contained several nuclei, up to as many as five.

The chief interest centres round the fact that certain varieties of chrysanthemum do not take the rust, though growing in the midst of plants thickly covered with it. One such variety which, while growing with rusted companions, I had examined and found clean, was chosen to experiment upon.

In the summer of last year leaves of this variety were inoculated with spores from a susceptible variety, and the inoculated spots were cut out and fixed each day, from the second to the tenth after inoculation. Sections of this material shewed that the germ-tubes had entered and had developed a mycelium with haustoria, just as in infection of a susceptible variety, though by the tenth day the mycelium was not as widely spread as in normal infection. The infected leaves which had been left on the plant did not develope uredospores; but by the fifteenth day (the normal period for the development of spores) the infected spots appeared as if corroded or scorched. These dead parts of the leaves on examination shewed a further stage of the same destruction of the leaf by the fungus, as is described later in the case of the leaves inoculated in the following summer.

The matter was at that time investigated no farther. In November inoculations were made on the leaves of flowering shoots, and nineteen days after inoculation uredospores appeared. A series of inoculations was then carried out, lasting till the end of March. These inoculations were made on flowering shoots, young shoots at the base of the plant, and on cuttings, but shewed no difference in result. Of thirteen sets of inoculations in all, uredospores developed in ten cases on some or all of the leaves inoculated; in the other three the inoculated parts of the leaves shewed the same scorched spots as in the Summer. The pustules which broke through were very small, but the uredospores were quite healthy and capable of infecting. Inoculations were made to see if these spores infected this variety more readily than spores from another variety, but no difference was found.

As it was possible that the unhealthy state of the plants (they had been growing in a hot greenhouse) caused them to take the rust, plants were inoculated which were somewhat starved but were otherwise quite healthy; the result however was the same.

The young plants were then divided into four groups. A were fed as if for exhibition, B were grown normally, C were starved by being grown in small pots, and D were grown in a warm greenhouse.

On inoculating them in July, about the thirteenth day after inoculation A shewed whitish flecks spreading over a great part of the leaves, B, C, and D shewed a paling of the leaves at the inoculated spots only. On hardening of the affected part of A and cutting sections of it the tissue of the leaf was found to be in a great part dead, the cells being shrunken till their walls almost touched, and the whole dead part staining very deeply. The mycelium was difficult to distinguish. The pale parts of B, C and D shewed the same features,

From the above we see that in an almost immune plant the capacity to feed the rust so that it develops normally does not depend on the state of health of the plant, but on the season of the year, though a luxuriant state of growth favours the spread of the mycelium.

Another species of Chrysanthemum *(C. Broussonetii)* was inoculated with rust from *C. sinensis*, and in this case the mycelium made little more headway than it did when *Ranunculus Ficaria* was used as host, but considerable destruction of the surrounding tissue took place. A very slight amount of killing was visible in the neighbourhood of many of the germ-tubes whose entry was recorded in the first part of the paper. So that it seems that whenever a germ-tube of any Uredine enters any plant but its own proper host a struggle goes on, resulting in the death of the host locally and of the parasite, and the more closely related the host is to the proper host of the fungus the more prolonged is the struggle and the wider the area covered by it, being greatest where the host is an immune variety of the proper host; while in normal infection both host and parasite continue to live in union for some long time.

An attempt to confer immunity by grafting failed. Shoots of a susceptible variety were grafted on to immune plants and after a junction was seen to have been made the new leaves which grew were inoculated, but these took the rust as freely as if the shoot were growing on its own roots.

I add some results on the temperature of germination and on the keeping power of spores.

Spores left for twenty-four hours in a temperature rising slowly from 0°—7°C shewed no germination. On being placed in a temperature of 20° they germinated freely.

At 6°—6·5° about half the sowing germinated at all temperatures from 7°—21°, free germination takes place.

At 21° rising to 25° all the spores germinated; but the tubes were curled and had wavy edges.

At 24°—25° never more than one-eighth of the sowing germinated.

At 30° no germination takes place and the spores will not germinate when removed to a lower temperature.

Kept dry at 35° for eighteen hours and them removed to a temperature of 17°, free germination took place.

Uredospores of chrysanthemum-rust were kept in a dry test-tube in a cool room from March 16th to May 26th, a period of seventy-one days. At that date about one quarter of the sowing germinated, but a week after none did so.

Aecidiospores of *Phragmidium rosae- alpinae* kept in the same conditions from May 20th till August 10th, a period of eighty-two days. On that date only one spore in a sowing germinated, but on July 28th quite half did so.

In conclusion I should like to express my gratitude to Professor Marshall Ward, at whose suggestion the work was begun and under whose supervision it has been carried out, and also to all those who have taken the trouble to collect various rusts for me.

DESCRIPTION OF FIGURES ON PLATES V. AND VI., ILLUSTRATING MISS GIBSON'S "NOTES ON INFECTION EXPERIMENTS WITH VARIOUS UREDINEÆ."

Fig. 1. Germ-tube of uredospore of *U. Geranii* entering stoma of *C. palustris* (2nd day).

Fig. 2. Under side of epidermis of *C. palustris* shewing substomatal swelling of germ-tube of uredospore of *P. Menthae* (2nd day).

Fig. 3. Germ-tube of Aecidiospore of *Uromyces Poae* entering *R. Ficaria* (2nd day).

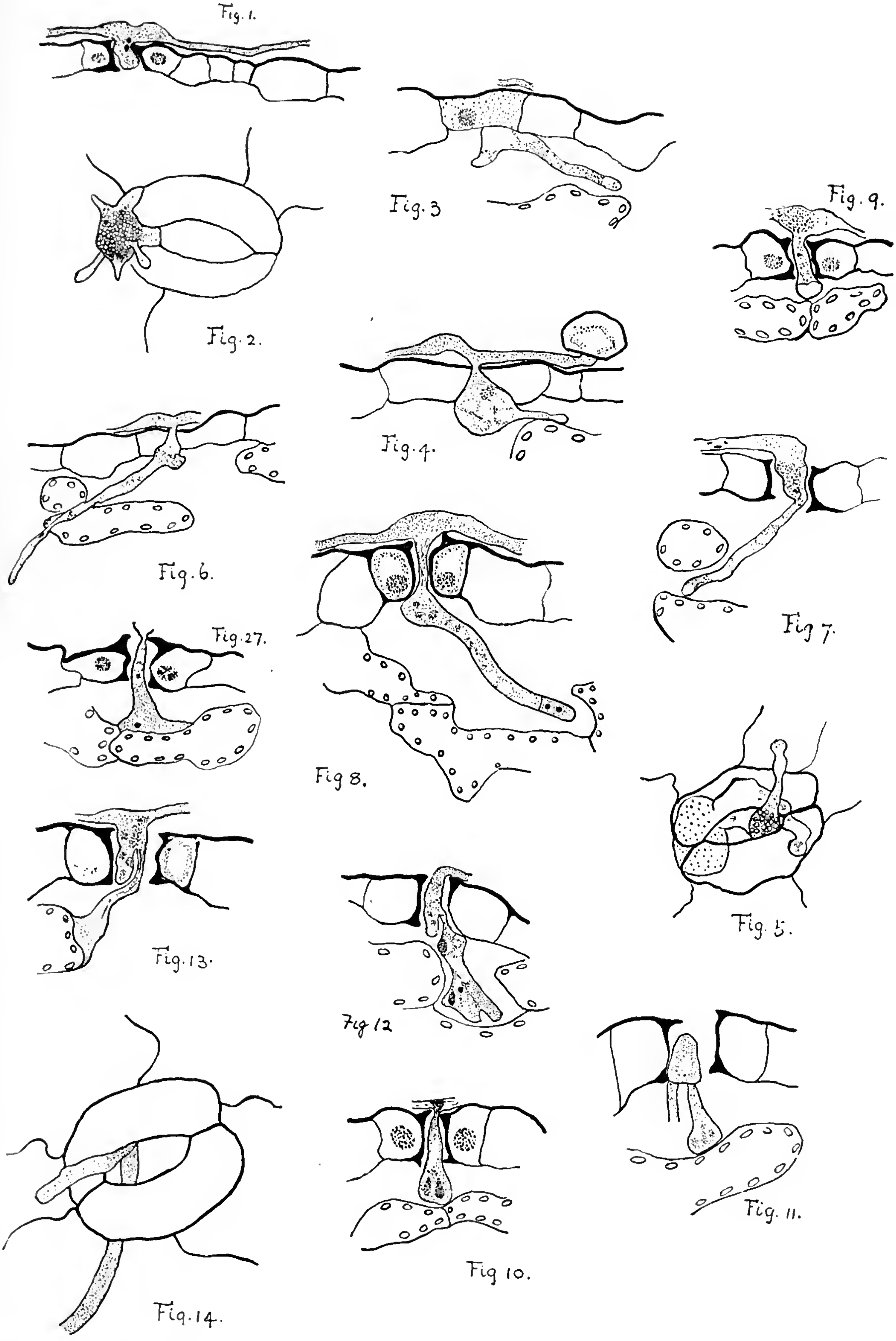
Fig. 4. Stoma, etc., of *C. palustris* shewing uredospore of *U. Geranii* germ-tube and sub-stomatal swelling emitting hypha (4th day).

Fig. 5. Under side of epidermis of *C. palustris* shewing uredospore of *U. Poae*, germ-tube and sub-stomatal swelling emitting hypha (2nd day).

Fig. 6. Germ-tube of uredospore of *U. Geranii* entering stoma of *C. palustris* and penetrating deep into mesophyll (4th day).

Fig. 7. Germ-tube from uredospore of *U. Geranii* entering stoma of *C. palustris* (2nd day).

Fig. 8. Germ-tube of uredospore of *U. Chrysanthemi* entering stoma of *R Ficaria* (3rd day).



GIBSON—INFECTION EXPERIMENTS WITH UREDINEÆ.

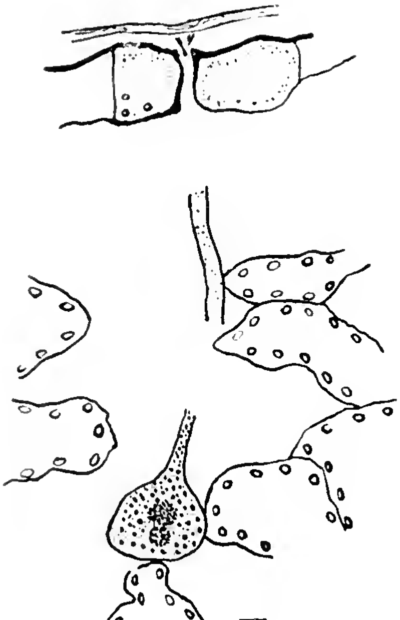


Fig. 15.

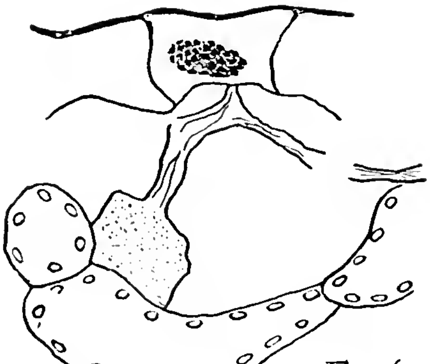


Fig. 16.

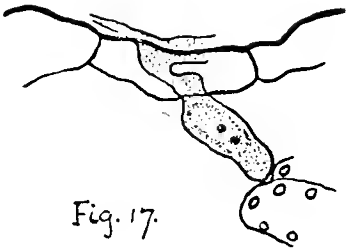


Fig. 17.

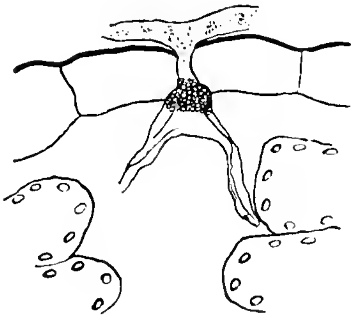
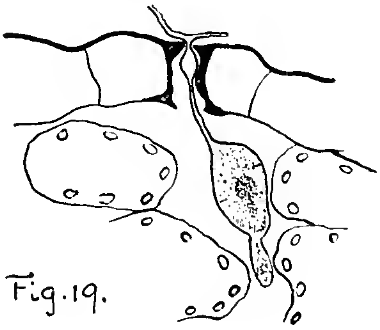


Fig. 20



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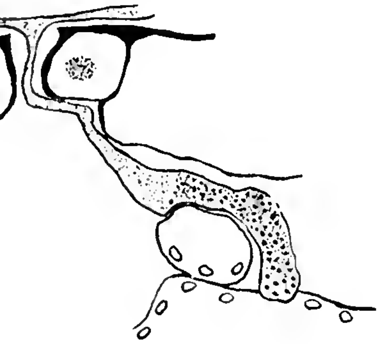


Fig. 18.

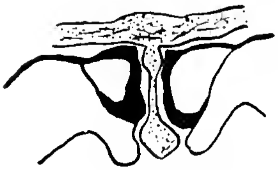
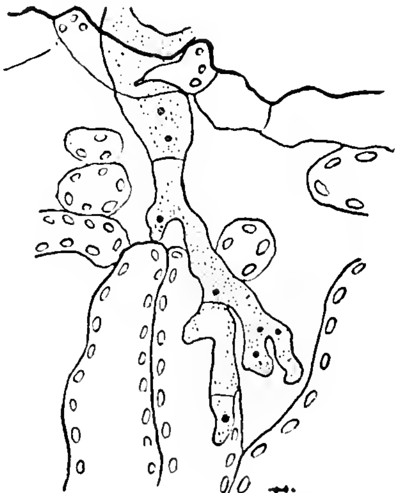


Fig. 21



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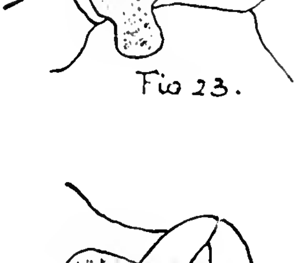

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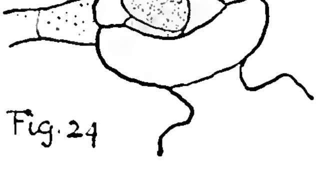

Fig. 24

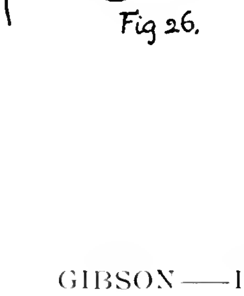

Fig. 26.

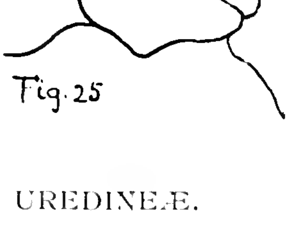

Fig. 25

GIBSON — INFECTION EXPERIMENTS WITH UREDINEÆ.

Fig. 9. Germ-tube of uredospore of *Puccinia* sp. from *Carduss* forming swelling in stoma of *C. palustris* (2nd day).

Fig. 10. Similar to Fig. 9 (2nd day).

Fig. 11. Germ-tube of aecidiospore of *P. Rosae* having formed a swelling in stoma of *R. Ficaria* has put forth two hyphae (3rd day).

Fig. 12. Stoma of *C. palustris* in which germ-tube of uredospore of *P. Poarum* has formed a swelling from which it has put forth a curious hypha (3rd day),

Fig. 13. Somewhat similar to Fig. 12, stoma being that of *R. Ficaria* and infecting fungus being *U. Chrysanthemi* (4th day).

Fig. 14. Stoma of *C. palustris* shewing germ-tube of uredospore of *P. glumarum* growing straight through without forming any swelling (2nd day).

Fig. 15. Germ-tube of uredospore of *U. Chrysanthemi* growing straight through stoma of *R. Ficaria* and forming swelling deep in the leaf (4th day).

Fig. 16. Somewhat similar to last, but swelling at a less depth and is already dead (4th day).

Fig. 17. Stoma of *Tropæolum* with germ-tube of *P. Taraxaci* growing through it, internal hypha much swollen (2nd day).

Fig. 18. Shews a similar hypha of *U. Chrysanthemi* which has penetrated the leaf of *R. Ficaria* to some depth (4th day).

Fig. 19. Germ-tube of *Puccinia* sp. from *Carduss* which has formed a somewhat deep-seated swelling in the mesophyll of *C. palustris* and has then emitted a hypha from it (2nd day).

Fig. 20. Germ-tube of *U. Geranii* entering *C. palustris*; the part outside is still alive. that within the leaf is dead (4th day).

Figs. 21 and 22 shew the different stage of development arrived at by two germ-tubes of *P. Centaureae* entering *Valeriana* (2nd day).

Fig. 23. Germ-tube of uredospore of *P. Menthae* entering stoma of *Valeriana* (2nd day).

Fig. 24. Germ-term of uredospore of *P. pulverulenta* entering stoma of *Valeriana* (3rd day).

Fig. 25. Germ-tube of uredospore of *P. graminis* entering stoma of *C. palustris* (2nd day).

Fig. 26. Germ-tube of uredospore of *C. Sonchi* entering stoma of *Valeriana* (2nd day).

Fig. 27. Germ-tube of aecidiospore of *P. Poarum* growing through stoma of *C. palustris* and forming swelling against a cell (3rd day).

THE PROBLEMS OF ECOLOGY,

BY A. G. TANSLEY.[1]

ECOLOGY may now be considered almost a fashionable study, but there are not wanting botanists who tacitly distrust, even if they do not openly contemn, a good deal of the work which is done under its name. This hostile attitude is not always wholly to be wondered at, for the subject has its own difficulties and dangers, and these are sometimes rather distressingly patent in

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[1] The substance of this essay was delivered as an Address to Section K of the British Association at the Cambridge meeting. It served as an introduction to the more special papers on British Ecology which occupied the rest of the sitting.

the papers of some of its votaries; on the other hand the hostility alluded to is not seldom due to misapprehension, and it therefore appears useful to consider the subject from a general point of view in the hope of pointing out some of its dangers and of clearing up some of the misapprehension.

In what follows the term "Ecology" is used in a somewhat narrower sense than is often the case. Instead of taking it as synonymous with "Bionomics" or "Natural History," to include all the vital relations of plants, as living and competitive organisms, with their surroundings and with one another, it will be restricted to what may be called the topographical aspect of these relations, or, in other words, to those relations which depend directly upon differences of habitat among plants. In this sense it is practically equivalent to the subject matter of Warming's book, a work that may be truly described by that often abused term "epoch-making," *Œcologische Pflanzengeographie*, or to that of Schimper's encyclopædic work *Pflanzengeographie auf physiologischen Grundlage.*

Though it may be open to question whether this use is strictly justifiable on logical or on etymological grounds, there can be no doubt that we want a single term for the subject matter so defined, as is sufficiently evidenced by the limitation of the subject matter of Schimper and Warming, and this is one which is simple and ready to hand. A single critical instance of the distinction to be drawn will make this limitation of subject matter perfectly clear. The study of individual pollination-mechanisms or of pollination-mechanisms in general would not be included as part of the subject-matter of ecology, since they do not directly depend upon any topographical factor; but, on the other hand, the general result arrived at by Hermann Müller in his *Alpenblumen*, that Alpine flowers are especially and pre-eminently adapted to pollination by Lepidoptera, as opposed to the bee- and fly-pollination prevalent in the lowlands, is an integral part of the ecology of Alpine plants, since a topographical factor clearly enters into this adaptation, the interrelation of the flowers and insects of the Alps as a habitat exhibiting characteristic life–conditions shewing a specialisation of its own.

As thus defined Ecology is simply the topographical physiology of plants, (or, as Schimper has it, Plant-Geography on a physiological basis)—using physiology in its wider sense to include all functional relations—and this is a fact which we shall do well to keep continually in mind.

When we survey the vegetation of the globe, the first great fact that meets us is its *diversity*. This diversity, however, is an *ordered* diversity. It is due in the first place, of course, to the enormous number of different species of plants that exist, but these species are not mixed haphazard upon the surface of the earth—they are associated in aggregates which are the result of perfectly definite and ascertainable, though often immensely complex, causes. Some of these causes may be called phylogenetic. Certain stocks or families of plants are confined to certain regions of the earth because their ancestors were evolved in those regions and have not succeeded in spreading beyond them. Thus many great families are exclusively tropical and subtropical e.g. the *Myrtaceæ*, the *Melastomaceæ*, the *Scitamineæ*. Others are mainly or exclusively temperate, e.g. *Ranunculaceæ*, *Papaveraceæ*, *Cruciferæ*, &c. There is no reason to assume that there is anything inherent in the constitution of the members of many of these families which prevents their spreading into those regions of the earth where they are not in fact found. We must rather suppose that having first appeared and obtained a footing in a certain region they have either not as yet had time to spread out of those regions, or (and this is probably the explanation in the majority of cases) they have failed to widen the limits of their distribution owing to the competition of the occupants of the other regions of the earth. In some cases, however, of highly specialised families, of which we may take the *Podostemaceæ* and the *Balanophoraceæ* as extreme instances, the members of the family have become so highly specialised to particular circumstances of life as to preclude the possibility of their spreading beyond the area of prevalence of these particular environmental conditions.

On the other hand many families are cosmopolitan. Among the lower forms of plant life, as for instance the Algæ and Fungi, not only is this true of the immense majority of the *families*, it is true also of many genera and species. The generic and even the specific identity of the algæ inhabiting the freshwaters of the Tropics with those of an English pond or stream is a remarkable fact, in striking contrast with the extreme rarity of generic identity between the members of the Phanerogamic floras of temperate and tropical regions. Here again we may distinguish two types of cause. In the case of some of the lower plants it may be that the immense time which has elapsed since they appeared on the surface of the earth has enabled them to spread over practically the whole globe, but the determining cause in the majority of cases is in all probability the ease with which they are distributed and the wide prevalence of the conditions under which they are able to exist. The same explanation holds in the case of the most widely spread genera of higher plants. Water plants are perhaps the most striking example. The conditions of life in water are far more uniform throughout the world than the conditions of life on land, while the ease with which aquatic plants are propagated vegetatively is well known, and these no doubt are the determining factors in the world-wide distribution of so many of the higher water plants.

Through these considerations we are naturally brought from the contemplation of what we may call *geographical* aggregates to those other aggregates which we may distinguish as *topographical*, and with which ecology in the sense we have defined, is mainly concerned.

In any region of the earth, in any country, in any limited area, we find the flora—composed as a whole partly of members of the families characteristic of the region, and partly of the members of cosmopolitan families – naturally falling into more or less definite "plant-associations" according to *habitat*. We find the plants of the mountain differing from those of the valley, the plants of marshy land differing from those of the dry plain, the plants of the coast differing from those of the interior, the plants of the sand-dunes differing from those of the salt-marshes. These differences are due to differences in the nature of the soil dependent largely upon the geological formations underlying the various areas; upon differences of water-content of the soil dependent partly upon the nature of the soil and partly upon the neighbourhood of large permanent bodies of water; upon differences of rainfall and of the humidity and movement of the air; upon differences of the salinity of the soil; and upon differences of temperature and illumination.

The morphological and physiological characters of the plants which make up the different associations found in these different environments are, as is well known, the result of *adaptation* to the conditions of life. Broadly speaking it may be said that only those species or individuals which possess, or are capable of developing under the appropriate stimulus, the special characters fitting them to exist under the given environmental conditions, are able to occupy and maintain themselves in the areas subjected to those conditions. The gross morphological features thus originating under the stress of a given set of particular conditions are often common to a number of different species and thus give to the association a definite *physiognomy*.

Now it is the study of such plant-associations, the species and individuals composing them, in their relations to one another and to their common environment which constitutes the main subject matter of *ecology*.

I think there is no general fact connected with plant-distribution which has struck me personally with greater force than the very close similarity of the plant-associations living in similar habitats in different parts of the globe. With certain notable exceptions the vegetation of the river-side, of the sea-shore, of the alpine summits of the tropics possesses exactly the same characteristics as that of a similar locality at home in Western Europe. In spite of the enormous differences of climate, *i.e.*, of temperature, rainfall and air humidity, the compelling effect of the topographical factor in forcing, so to speak, the vegetation found in similar types of habitat along parallel lines, into parallel associations, is everywhere seen.

It is obvious that the study of such associations falls under two heads, corresponding to the two stages of procedure inevitable in natural science. First, the mainly descriptive stage. The plant-associations which are the objects of our study must first be characterised, enumerated, and described. The different species making up an association must be catalogued, their obvious relations described, the physical conditions under which they exist taken note of. The variations of each association must be recorded, as well as the transitions from one to another. This is the business of *ecological survey*. Without it, our knowledge, regarded merely from the descriptive standpoint, of the flora of a country or region of the earth, is entirely one-sided and inadequate. We cannot content ourselves merely with the knowledge that certain species of plants occur. We must know exactly how they occur, in what situations, associated with what other plants, before we can have a complete mental picture of the vegetation. Such knowledge is as theoretically justifiable and necessary as, and, in fact, is in every respect comparable with, that gained by a geological survey and the construction of geological maps. The philosophers tell us that the first object of that human curiosity which is the basis of all science is to "intuit," *i.e.*, to make a direct mental picture of, the contents of space and time. We cannot intuit the contents of space botanically without the method of ecological survey. In the old days, when vast tracts of the earth's surface were still unexplored, when botanical collecting in a new country meant that the great majority of species brought home were still undescribed, it was natural that the collection and description of new species should be the first object of the botanical explorer. Even then something was done by the more intelligent collectors in the way of description of the vegetation as a whole, of the associations in which the species were found. But now, when the vast majority of species of flowering plants at least have been described, our attention must be more and more turned towards acquiring a knowledge of the associations or combinations in which plants occur. Even in the countries of Western Europe and America, the home of the races by whom the study of plants is mainly cultivated, we are still very far from an approximately complete knowledge of our plant-associations; though the work of Warming in Denmark, Flahault and his pupils in France, Graebner and others in Germany, many botanists in America, and quite recently several in Great Britain, especially in the north of England and in Scotland, has done and is doing something to take away this reproach. Schimper's great work to which I have already alluded is a magnificent encyclopædia of what was known at the date of its publication of the plant-associations throughout the world, knowledge of which his own investigations on tropical floras contributed no small part.

But we cannot, of course, stop at this first stage. The philosophers go on to tell us that the second and highest goal of scientific curiosity is to unravel the *causes* of phenomena, to go beyond the intuition of the gross appearances of what we see around us, to enquire as to their causation, or, to speak more precisely, to attempt the determination of the simple invariable time-sequences which exist in nature.

When we consider the application of this principle to our ecology or topographical physiology, we see that it involves an attempt to determine *why* the plants which live together on a definite area with definite environmental conditions come to be thus associated—how they come to be and how they maintain themselves where they are, how they come to exhibit the morphological and physiological features they do exhibit, and what are their detailed functional relations to one another and to their inorganic surroundings. These indeed are topics on which our knowledge is of the most fragmentary description, about which we have still practically everything to learn. It is a much more difficult, as it is undoubtedly a much higher task, than the descriptive one. It involves careful and patient observation and experiment, and the application and adaptation of the methods of ordinary physiology to the solution of these special problems.

It is obvious that the two stages of ecological study, the descriptive and the experimental, need not be and should not be completely separated in practice, though they may be logically distinct. So far as possible the descriptive study of plant-associations should go hand-in-hand with enquiries into the causation of the phenomena involved, but it must be admitted that this ideal union is not always a practicable one. In the case of the botanical exploration of a new country, it is clearly out of the question for the traveller to do much more than collect and record the associations he meets with. He is fortunate if he is able to make a few hasty observations on temperature, atmospheric humidity and the like, while it is obvious that no systematic experimental work can be undertaken. Yet his collections and records, if intelligently made, are of the greatest use in filling up gaps in our knowledge of world-vegetation. And the same is true I think even of our own country. Here, of course, the resident recorder of the broad features of the vegetation has great advantages over the travelling recorder. He has time to prepare vegetation-maps of any degree of accuracy and detail that seems called for, and he can visit his areas over and over again, noting not only seasonal, but progressive secular changes in the flora. He has leisure to make systematic observations on soil, temperature and rainfall, and to determine their relations with the distribution of vegetation. Botanical survey work of this kind, such as has been extensively carried out on the practically untouched mountain and moorland plant-associations of Scotland and the north of England during the last few years, is of great value, quite apart from that experimental investigation which everyone will admit is required to elucidate completely the ecological relations of plant life. Broad problems arise which can be solved only by extensive comparative work in the field, and until we go into the field and work out the distribution of vegetation systematically, we may not even suspect their existence. The experimental investigator confining himself to a limited flora will necessarily miss altogether unsuspected combinations of plants which the surveyor and cartographer of large areas meets with.

The importance of map-making as a graphic record of the distribution of vegetation is very great. It as necessary to the formation of clear mental pictures of the facts of distribution as geological maps are in the distribution of rocks and drift-deposits; and such clear mental pictures are essential to the appreciation of the problems involved. The making of adequate botanical maps is not an easy or a straightforward task, as anyone who has seriously tried it knows very well. The science of botanical cartography is yet in its infancy, and the pioneer work of Drude, Flahault and the Smiths has only begun to shew us its possibilities. The various difficulties that surround it cannot be entered upon here, but one point of importance may be mentioned. The vegetation of an area cannot be presented once for all on a single map. Different maps are required for bringing out different aspects of the flora, and maps on different scales are absolutely essential. A scale suitable for exhibiting the main features of the vegetation of a given region often requires supplementing by larger scale maps of portions of the region to shew more detail. Again a scale adequate to the display of the leading vegetation-features of one region may shew nothing worth shewing in another part of the country.

For these reasons I should like to see a central committee formed for the systematic survey and mapping of the British Isles. The time necessarily taken by the travelling and field work makes it most desirable that such work should be planned on a comprehensive scale and the proper co-ordination of the results obtained in different parts of the country makes co-operation, frequent consultation of different workers, and I think, in the case of the less experienced, some kind of central direction, of great importance. A further point which such a central committee might hope to deal with, and one which if dealt with successfully would do much to remove what I have always thought is one of the most crying examples of the waste of good work and sound knowledge in the field of modern botany, is the utilisation of the work of the local botanist and the local field club. Scattered up and down the country are scores of men whose hobby is botany and whose acquaintance with their local floras is absolutely unequalled. Too often they carry with them to their graves knowledge which would be of the greatest value in helping to build up a picture of the vegetation of the country as a whole. Convince them of the interest of ecological survey work, and you would secure their co-operation in working out and mapping local floras from that point of view, which with the requisite general knowledge of methods and a certain amount of help and direction, they would do a hundred times better than a visiting botanist, with no knowledge of the locality. The extensive co-operation and co-ordination of workers which might result in the course of a few years would probably lead to a very striking development of the whole subject.

A real danger to which workers in this field are exposed could be largely avoided by properly organised work under a general scheme. The great variation in detail of plant-associations, which is a result of the very complex factors combining to produce the vegetation of an area as we actually see it, gives no doubt ample opportunity for the waste of time over recording details of trifling importance, and this danger is all the greater because the higher scientific development of the subject is still in such a rudimentary condition that we are frequently quite in the dark as to what *is* of importance and what is not. The danger I have thus briefly touched upon has not been without its exemplification in some of the work that has been published, and has been responsible for much of the distrust of ecological work to which reference was made at the outset.

Another type of criticism which has been made, though superficially plausible, is I venture to think largely due to misapprehension. It has been objected that the straight-forward mapping of the vegetation of an area without the previous formulation of definite problems for solution is a mistaken and unscientific procedure. I have already attempted to shew that it is, on the contrary, a legitimate and necessary part of our attempt to become completely acquainted with the vegetation of the world, and that if we neglect it and content ourselves with the floristic system we obtain a one-sided and misleading knowledge. Till a certain number of accurately observed facts reveal themselves we cannot possibly formulate problems. There can be no problems in comparative anatomy till we know the structure of a certain number of organisms, nor in stratigraphical geology till we know the distribution and succession of a certain number of rocks. And similarly there cannot be problems in the study of plant-associations till we know the nature and distribution of the associations in question. The problems arise, and are arising, fast enough as the facts are ascertained. And that must be so in all branches of science. We must remember that the systematic study of ecology is no more advanced than plant-anatomy was in the time of Von Mohl, or geology before Lyell, Sedgwick and Murchison; though no doubt it differs from these subjects in being a kind of synthesis of several branches of science, rather than a new branch having to develop from the ground. It is possible, of course, to go on aimlessly piling up facts with little thought of what they may mean. It is possible to adopt a method unsuitable for the region chosen and thus fail to obtain satisfactory results. But these are dangers not peculiar to ecology, but rather to the unintelligent pursuit of any branch of knowledge.

On what I have called the second stage of ecological work, the experimental enquiry into the complex relations obtaining between the plants making up an association and their environment, there is no space to enlarge. Starting points for such enquiries are constantly revealing themselves during the ordinary work of surveying, but it is not everyone that has the opportunity or the skill to undertake them. A good deal of scattered work in this field has of course been done by various botanists, whom it would be superfluous to name. One of the most important means of progress in these higher branches of ecology is undoubtedly the establishment of laboratories in regions exhibiting specialised types of vegetation. The most recent and one of the most notable examples of such a laboratory is the Desert Laboratory of the Carnegie Institution, established in November, 1903, near Tucson, Arizona, under the direction of Dr. Coville, whose researches on Desert vegetation, published in the Report of the Death Valley Expedition, are already well-known to ecologists. The Tucson laboratory site was chosen after a careful tour through the North American deserts by Drs. MacDougall and Coville. Not only the Director but visiting botanists able to make a stay, will work at the ecological relations of the desert flora.

We have no deserts in the British Isles, but we have a fair range of climatic conditions and a very interesting set of "edaphic" plant formations. In the future we may perhaps hope to see various local stations established where experimental work may be carried on.

A SECOND EXPERIMENT IN ECOLOGICAL SURVEYING.

A SHORT account was given in this journal (Vol. II., p. 167) of an attempt made in July, 1903 to study the vegetation of a given area by an expedition from the Botanical Department of University College, London, composed of the staff and a number of advanced students.

That expedition was to the "Broad" district of East Norfolk, and was devoted partly to the magnificent fresh-water marsh-vegetation characteristic of the region and partly to the sand-dunes fringing the coast. Many difficulties were met with. The time available was scarcely long enough to map even a small typical area satisfactorily, and the difficulties of getting about on the deep marsh were considerable; while the fact that the locality chosen (Heigham Sound) is very strictly preserved led to some reluctance on the part of the owner in allowing access to it. Nevertheless the expedition was certainly successful in many respects. Its members learned a great deal of the marsh-plants and their habits, and some interesting data were obtained, which though not sufficiently complete to publish at present, it is hoped to extend and use at some future time.

In the present year an expedition lasting a fortnight (Aug. 27th to Sept. 10th) was organised on a considerably larger scale. Advantage was taken of the experience gained last year, and an area was chosen, easy to get about on, and with a very sharply defined characteristic flora consisting of comparatively few species, whose distribution was evidently largely dependent on certain physical relations. The area in question is the estuary of a small stream, known as the Bouche d 'Erquy, on the north coast of Brittany, about twenty-five miles west of St. Malo. The estuary itself, about 150 acres in extent, is separated from the sea by a spit of old sand-dunes, which extends at right angles to the main direction of the river, and allows it egress by a comparatively narrow channel. The lower part of the estuary is covered with bare sand brought in by the tide and constantly shifting in position, but practically the whole of the upper part is occupied by a carpet of halophilous vegetation. The soil here is largely mud, though this is over-laid in places by sand brought up by the tide, and surface borings reveal constant alternations of sand and mud-layers. The highest parts of the estuary are only covered by water at the highest spring tides, *i.e.*, for very few hours in the month. Rather lower tides cover more or less of its surface, while the lowest neaps are barely felt in the channel of the stream. There are three small tributary streams running through the estuary and emptying themselves into the main river; their channels, carved out and scoured by the tide, are cut through the gently undulating plateau which forms the general surface of the estuary and lies a few feet above the surface of the stream-beds. This plateau is also intersected by channels of varying depth, which do not serve for the passage of streams, but up which the higher tides run.

The plateau is covered by plant associations, in which five species are dominant:—*Salicornia herbacea*, *Glyceria maritima*, *Suaeda fruticosa*, *Obione portulacoides* and *Juncus maritimus* These five form the great bulk of the vegetation, while associated with them are *Salicornia radicans*, *Spergularia marina*, *Aster Tripolium*, *Armeria vulgaris*, *Statice Limonium* and others. The distribution of the associations, as has been said, is obviously dependent very largely on differences of level, but there are clearly several factors at work, and their elucidation presents problems of great interest. A very marked feature of the vegetation when it is at its highest point of development, in August and September, is the colour of the *Salicornia* and *Suaeda*. These plants vary from bright green to deep crimson, according to their place of growth, and the landscape effects produced are of very striking beauty.

The immediate object of this year's Expedition was to map and level the physical features and the plant-associations of the area, while concurrent work was to be carried on upon the nature and wetness of the soil, salt percentages, etc. It will be seen that the area presents great attractions to the botanical cartographer. The plant-associations are very well marked, and, with the exception of the comparatively slight influence produced by the grazing of a few sheep and cattle, is practically untouched by human agency. The surface dries rapidly enough to make it easy to walk about upon except during the few hours per month when it is actually covered by the tide, and the differences of level, while of great importance to the distribution of the associations, are not great enough to put difficulties in the way of the actual work of surveying.

It was originally intended to work on the lines of an ordinary land survey, but after various preliminary field experiments near London, it became clear that some modification of this method was very desirable. The problem being to map the details of complicated areas of vegetation, ordinary survey lines and off-sets become unworkable; for either the number of lines has to be multiplied to an impracticable extent, or the individual off-sets have to "pick up" so many boundaries that confusion inevitably results.

The solution was found in the "method of squares." A convenient base line is taken, as in an ordinary land survey, and upon this rectangles are constructed on each side so as to enclose the whole of the area it is desired to map. Flagged sticks are placed at intervals of 100 feet along the base line and along the sides of these rectangles. The whole area of each rectangle is then plotted out into squares with sides of 100 feet each by placing other flagged sticks at intervals of 100 feet each way, and this is done by simple ranging against the flags of the base line, perpendiculars, and parallels.

The work of mapping now begins. The party is divided into several sections to each of which one or more rectangles is allotted. Each surveyor is provided with a field-book consisting of leaves of squared paper, and each 100 foot square is mapped on a single leaf. The physical features are put in first and then the boundaries of the different plant-associations, those which are to be recognised having been previously agreed upon and designated by symbols. Any point within the square can be at once identified in the map by means of imaginary perpendiculars dropped from known points on the sides, so that the surveyor, by standing at the point he wishes to mark, can get two assistants to fix his position by optical squaring from the side lines. In practice, by placing sticks at intervals of 20 feet along the side lines before he begins to map, the surveyor can soon judge his position in the square with sufficient accuracy, without resorting to the use of the optical square. After salient points are marked the actual boundaries can be rapidly drawn by eye with considerable accuracy. Meanwhile detailed notes are made on the vegetation included in the square, and representative plants are collected, labelled, and pressed.

In this way a map of the whole area was constructed in sections on a scale of $\frac{1}{240}$ and a detailed record of the vegetation obtained. This map is now being put together and reduced.

As has been indicated above, the halophilous vegetation of the Bouche d' Erquy is evidently closely dependent on very slight changes of level, and these changes constantly occur in the course of a few feet or even of a few inches. It therefore became desirable to map certain characteristic portions of the area on a larger scale than that adopted in the general map. This was done by means of the "gridiron" method, which essentially consists in taking a 25-foot square, laying tapes on every side, and also laying four other 25-foot tapes across the square parallel to the sides, at intervals of 5 feet. A plan is then constructed on squared paper, as in the method of squares, but on a scale of $\frac{1}{60}$. Details correct to less than 6 inches can be mapped on this plan with great rapidity, since the surveyor can always stand immediately over his work. Levels are then taken with a telescope and levelling staff reading to $\frac{1}{100}$-foot, wherever desired, so that contour-lines $\frac{1}{10}$-foot apart, which are of great importance in attempting to understand the distribution of the vegetation, can afterwards be constructed. A full account of the "gridiron" method will appear in an early number of this journal.

A great deal of time and thought was spent on the organisation of the work of surveying, and it was most satisfactory to find in the field that this labour was by no means thrown away. It would indeed have been impossible to get through the work without the careful organisation employed. The party consisted of about twenty-seven persons, mostly from University College, but with representatives from Cambridge and from the Royal College of Science. The bulk of the party was divided into five working sections of three members each, who undertook respectively the duties of Surveyor, Diarist, and Collector, the Diarist and Collector helping the Surveyor when necessary. To each section a given area was assigned, after the preliminary work of constructing a base-line with the necessary perpendiculars and parallels, and of ranging the squares, was completed. The rest of the party formed the Staff, and its members undertook this preliminary work together with the general direction of the detailed survey, as well as the earth-boring, levelling, salt-estimations, photographing characteristic samples of vegetation, etc. Two artists, who devoted themselves to sketching in water-colour characteristic salt-marsh landscapes, also joined the party. During the progress of the work it became apparent that some of these departments, particularly the estimations of salt and wetness of soil, should have been more carefully organised, and it is hoped to return to the area next year with the object of obtaining far more complete data on these points. Meanwhile the construction of the map and the correlation of the data already obtained will be proceeded with, and in the light of the results arrived at, an organised scheme for the supplementary work required will be drawn up. The "gridiron" work was mostly carried out by six members of the party who remained for some days after the end of the fortnight.

The expedition was by no means devoid of more or less humorous incident. Permission was obtained from the French Government, through the Foreign Office and the British Embassy in Paris, to carry on the work of surveying. Owing to an error in the transmission of instructions from Paris, the mayor of one of the communes in which the estuary is situated, refused to allow the work to proceed on one side of the river, and this necessitated a certain re-adjustment of arrangements, and much telegraphing, before progress could be made. Subsequently some rather officious French visitors to a neighbouring resort conceived it to be their duty to interfere and protest against the evidently nefarious designs of the party upon the well-being of the Republic, cunningly concealed by the pretext of botanical investigation. The connexion of surveying and levelling instruments with Botany was, not unnaturally, far from obvious to their apprehension. The local officials were, however, able to set the minds of these anxious patriots at rest, and the party proceeded without further interruption, beyond the inconvenience caused by the irresistable fascination which the pulling up of flagged sticks exercised upon some of the younger members of the local peasantry and fisher-folk. The opportunity of acquiring for nothing a number of fine bamboo poles was also not without its attraction to some of their elders. These experiences, however, though rather disconcerting, could no doubt be avoided by a little judicious arrangement beforehand. For the most part the expedition met with much kindness and courtesy from the local officials and population.

The experiment, in its way rather ambitious, may be said, on the whole, to have been an unequivocal success.

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STUDIES IN THE MORPHOLOGY OF *TORREYA CALIFORNICA*, TORREY.[1]

II.—THE SEXUAL ORGANS AND FERTILISATION.

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[WITH PLATES VII., VIII. AND IX.]

I.—INTRODUCTION.

THE observations recorded in the present paper form a continuation to a study of the early history of the reproductive organs of *Torreya californica*, which was published in THE NEW PHYTOLOGIST in July last.[1] References will be found there to previous papers on this plant. A long series of hand sections illustrating the development of the ovule was cut by Mrs. Tansley from fresh and spirit material collected in 1902, and she obtained preparations shewing the archegonia, and embryos, and in at least one case the actual entry of the male nucleus into the egg. These preparatious were handed over to me, and I have found them of the greatest possible use. They were especially invaluable in determining probable dates for the critical stages, and hence in deciding when it would be advisable to fix the ovules on the spot. The material, as before, was obtained from the beautiful tree in the Orton Longueville gardens, and I have to thank Lord Huntly for his kindness in not only placing the material at my disposal, but in allowing me to collect the ovules myself, and fix them at the tree during August and September. I am also indebted to Mr. A. Harding, the head gardener, for numerous consignments of material.

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[1] Studies in the Morphology of *Torreya californica*, Torrey. I.—"Spore Formation in *Torreya californica*." THE NEW PHYTOLOGIST, Vol. III., 1904, p. 133. The general title did not appear in the heading of the first paper.

I have great pleasure in thanking Professor Oliver, at whose suggestion the work was undertaken, for the help and advice which he has most kindly given me throughout.

I hope in a future paper to describe the structure of the seed in its second year of development.

II.—METHODS.

During the seven weeks from August 3rd to September 19th of this year material was fixed at the tree, as a rule once in three days. To ensure good penetration of the fixing fluid, the four thick green bracts which surround each ovule, and the free part of the arillus, were removed with a scalpel, and the nucellus laid bare as completely as possible by cutting away the adherent part of arillus and integument. The small conical cap formed by the free apex of the integument was generally removed with a needle. Flemming's weak solution was the fixing fluid chiefly used, but Flemming's strong solution, weak and strong chrom-acetic solutions, 1% chromic acid, Juel's fixative, and absolute alcohol were also employed to some extent. *Flemming's weak solution* followed by Flemming's triple stain, and *Juel's fixative*, followed by either Heidenhain's haematoxylin or Flemming's triple stain, gave much the best results. Juel's fixative[1] (2 grs. zinc chloride, 2 cc. glacial acetic acid, 100 cc. 45-50% alcohol) is particularly convenient for use, as it is a clear liquid unaffected by light. Material fixed in Flemming's strong solution, or strong chrom-acetic, was very apt to crumble to pieces in the paraffin. Except in the case of absolute alcohol, the dissected ovules were suspended at once in small muslin bags in a jar of the fixative, where they were left for twenty-four hours. After solutions containing chromic acid the ovules were washed for a day with many changes of water, and then passed up in the dark through graded alcohols to methylated spirit, and thence to Calberla's fluid (alcohol-water-glycerine) where they were left until needed. The dehydrated fragments were penetrated with bergamot oil and embedded in 52° paraffin. The sections were cut with the microtome as a rule to a thickness of 10μ.

III.—THE FEMALE PROTHALLUS.

The concluding figure of the first part of this paper shewed a uni-nucleate embryo-sac capped by three disorganising sister spores (l.c. Pl. iv., Fig. 32). In an ovule gathered on June 30th, a week

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[1] H. O. Juel. "Ueber den Pollenschlauch von Cupressus," Flora, 1904, p. 56.

later, the first stage in the germination of the megaspore had been reached, and the embryo-sac was bi-nucleate (Fig. 1.) Four-nucleate and eight-nucleate embryo-sacs have also been found, the latter occurring on July 6th. Later on numerous free nuclei are seen in the protoplasm lining the embryo-sac. Wall formation seems to proceed by in-growing tubes as described by Mlle. Sokolowa[1] for so many Gymnosperms. In two cases I have seen the alveoli from opposite sides of the embryo-sac approaching the centre, but not yet meeting. In one of these cases archegonia were already present. Mlle. Sokolowa says that *Cephalotaxus* is exceptional in that the partitioning of the alveoli begins before they have met in the middle region, and in this point *Torreya* agrees with *Cephalotaxus*.

Jäger[2] mentions that in *Taxus* there is an axial core of cells which are smaller and richer in protoplasm than the rest, and have conspicuous nuclei. He also describes the cells at the base of the endosperm as small, rich in protoplasm, and meristematic, but he does not speak of them or figure them as forming a distinct layer. In *Torreya californica* there is no special axial core, but about the middle of August differentiation begins to occur in the basal region. The outermost layer of the endosperm gradually becomes distinguishable from the rest by its conspicuous nuclei and contents and its meristematic character (Fig. 2). The nucleus is generally centrally placed and suspended by protoplasmic filaments (Fig. 3). When sections of an ovule gathered at the end of August were treated with iodine it was found that this layer was rich in starch while the rest of the endosperm was almost or quite starch-free. In sections of fresh material collected at the end of September the layer very conspicuous. It was one cell thick through most of its extent, and reached rather less than half-way up the endosperm, enclosing the rest of the tissue in a kind of cup (Fig. 28). During the growth of the prothallus which takes place chiefly in the downward direction (cf. Figs. 4 and 28) the surrounding nucellar tissues become crushed and apparently absorbed (Fig. 2). It seems possible that the outer layer of the base of the prothallus may be of an epithelial nature, and secrete a ferment. In that case the growth of the prothallus through the nucellus would be comparable with the growth of an endogenous root enveloped in its "digestive sac."

[1] C. Sokolowa. "Naissance de l'endosperme dans le sac embryonnaire de quelques Gymnospermes." Bull. de la Soc. Imp. des Nat. de Moscou., 1890, p. 446.

[2] L. Jäger. "Beiträge zur Kenntniss der Endospermbildung und zur Embryologie von Taxus baccata." Flora, 1899, p. 241.

IV.—THE ARCHEGONIA.

Early in August the young archegonia have begun to develope. Figs. 4 and 5 shew an ovule containing one with a two-celled neck. On August 12th, six days later, an archegonium was found with one of its two neck-cells undivided, and the other with two daughter-nuclei at the poles of a spindle (Figs. 6 and 6a). The number of neck-cells in the mature archegonium seems to be somewhat variable. Figs. 7 and 8 shew transverse sections of necks which are respectively four and six-celled. There is normally only one tier, but occasionally one cell may divide by a periclinal wall as shewn in longitudinal section in Fig. 9. The number of archegonia in each ovule is two, three or four, three being the number most commonly met with (Fig. 10), and I have once seen as many as five. In *Taxus*, Jäger[1] speaks of five to eight as the usual number. The archegonia are usually placed at the top of the prothallus, but three times I have found one in an abnormal position quite low down. I have once found a double archegonium resembling those several times observed by Miyake in *Picea*.[2]

The jacket cells are rather richer in protoplasm than their neighbours and have conspicuous nuclei (Fig. 11), but I have not observed any pits in their walls, or transference of nuclear matter into the archegonium as described by Arnoldi[3] for various Conifers.

In late stages the archegonia become buried some little distance beneath the surface of the prothallus, recalling Jäger's description of *Taxus*.[4]

Of half-a-dozen ovules gathered on August 20th, five shewed archegonia with the nucleus of the central cell undivided (Fig. 11). The sixth contained three archegonia, of which two shewed the nucleus of the central cell in the act of dividing to produce the ventral canal and egg-nucleus. One pollen-tube had entered the nucellus, but had not yet reached the embryo-sac. It ran nearly in the plane in which lay the archegonium which was the more advanced in the division of its nucleus. The chromosomes of the two daughter-nuclei were slender and V-shaped (Fig. 13), recalling Coker's figure of this stage in *Taxodium*.[5] It is remarkable that in

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[1] L. Jäger. Loc. cit.

[2] K. Miyake. "On the Development of the Sexual Organs and Fertilisation in *Picea excelsa*." Annals of Botany, Vol. XVII., March, 1903.

[3] W. Arnoldi. "Was sind die Keimbläschen." Flora, 1900, p. 194.

[4] L. Jäger. Loc. cit.

[5] W. C. Coker. "On the Gametophytes and Embryo of *Taxodium*." Bot. Gaz., July, 1903, Fig. 87.

no other ovule have I found any certain trace of a ventral canal-nucleus. There is often in the later stages a curiously reticulate and fibrous structure, sometimes including small safranin-staining bodies, in the protoplasm of the tip of the egg-cell; this may possibly be the result of disintegration of the ventral canal-nucleus (Fig. 14).

During the latter half of August the egg-nucleus grows a good deal, but its chromatin does not appear to increase, and tends to concentrate in the middle of the nucleus. One or more nucleoli are present. The rest of the nucleus is filled with a finely granular substance. Fig. 14 shews the state of an egg-nucleus early in September. When the actual union of the male and female nuclei is taking place, the chromatin is againdistributed fairly evenly through the egg-nucleus (Fig. 20). The protoplasm of the mature archegonium is vacuolated and granular and often contains one or more darkly-staining masses near its base (Fig. 20). The presence of these deeply staining structures in the protoplasm of the egg-cell has been noted in various Conifers e.g. *Thuja*.[1]

V.—THE POLLEN-TUBE.

I have not found any indication of a pollen-chamber in the apex of the nucellus. No prothallial cells are produced in the pollen-grain, which consists when ripe of two cells, of which presumably the smaller is the generative-cell and the larger the tube-cell. The youngest pollen-tube which I have been able to observe occurred in an ovule whose embryo-sac was eight-nucleate, and here the division of the generative-cell had already taken place. A pollen-tube at a similar stage is drawn in Fig. 12. Both the body-cell and its nucleus increase greatly in size, and Fig. 15 shews the end of a pollen-tube from an ovule collected seven weeks later. Here the stalk and tube-nuclei are lying closely pressed together in front of the body-cell. In later stages only one vegetative nucleus can as a rule be recognised in the pollen-tube, and this may be brought about either by the fusion of the stalk and tube-nuclei, or by the disappearance of one, probably the tube-nucleus. A pollen-tube at a similar state of development to that shewn in Fig. 15 was tested for starch, which was found to be present in the protoplasm surrounding the stalk and tube-nuclei, but could not be recognised in the dense substance of the body-cell. The protoplasm of the body-cell at this stage shews slight radiations from a point in front of the

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[1] W. J. G. Land. "A Morphological Study of *Thuja*." Bot. Gaz. XXXVI, 1902.

nucleus, suggesting, though to a less degree, the curious structure found by Miss Ferguson[1] in the cytoplasm of the body-cell of *Pinus* before division of the nucleus. I am sorry to say that I have not succeeded in observing the division of the body-cell nucleus. Fig. 16 shews a body-cell enclosing two sperm-nuclei. These two nuclei seem never to be surrounded by separate masses of cytoplasm, agreeing in this respect with *Pinus*.[1] Fig. 17 shews one male nucleus in contact with the neck of an archegonium while the second is some distance behind. One vegetative nucleus is in contact with the body-cell. I believe that only one of the two male nuclei is functional, for I have invariably found a separate pollen-tube corresponding to each fertilised egg. So far as I have observed the functional nucleus is no larger than the other. In the equality of the sperm-nuclei *Torreya* agrees with *Cephalotaxus* as described by Arnoldi,[2] but differs from *Taxus*[3] as represented in Belajeff's well-known figure.

VI.—Fertilisation.

The passage of the functional male nucleus into the archegonium is shewn in Fig. 18, which is drawn from an ovule gathered on August 31st. The second male nucleus and the rear half of the body-cell are left in the pollen-tube. A hand section, cut by Mrs. Tansley, of an ovule collected on September 17th, 1902, shews a similar stage. In Fig. 19 the male nucleus is actually in contact with the egg-nucleus whose membrane is pressed in like a collapsing india-rubber ball. This peculiar method of union of the male and female nuclei was first recorded by Blackman[4] for *Pinus*, and has since been demonstrated in various other Gymnosperms. It appears usual in Conifers for the male nucleus to slip from its protoplasmic sheath as it approaches the egg-nucleus, and leave it behind near the point of entrance. In *Sequoia*[5] the male nucleus even enters the egg naked, shedding its cytoplasm outside. Coker[6] in his paper

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[1] M. C. Ferguson. "Development of the Pollen-Tube and the Division of the Generative Nucleus in Pines." Annals of Botany. Vol. XV., 1901, p. 194.

[2] W. Arnoldi. "Embryogenie von Cephalotaxus Fortunei." Flora 87, 1900, p. 46.

[3] W. C. Belajeff. "Zur Lehre von dem Pollenschlauche der Gymnospermen." Ber. der deutsch. Bot. Gesellschaft. Bd. IX., 1891, p. 280, Pl. XVIII., Fig. 13.

[4] V. H. Blackman. "On the Cytological Features of Fertilisation and Related Phenomena in *Pinus silvestris*, L." Phil. Trans. Roy. Soc. 190 B., 1898.

[5] A. A. Lawson. "The Gametophytes, Archegonia, Fertilisation, and Embryo of *Sequoia sempervirens*." Annals of Botany, Vol. XVIII., 1904, p. 1.

[6] W. C. Coker. Loc. cit.

on *Taxodium* was the first to describe a case in which the cytoplasm of the male nucleus surrounds the fusion-nucleus and plays an important part in the formation of the embryo. In this plant the male cytoplasm is packed with starch. A similar case has recently been recorded by Lawson for *Cryptomeria*.[1] Although Jäger does not refer to it in *Taxus*, two of his figures (Pl. xvii., figs. 38 and 39) distinctly suggest that the same thing takes place in this genus. I have observed the contact of the male and female nuclei in seven archegonia of *Torreya californica*, and always without exception a layer of specially dense cytoplasm has been found to occur on the side of the two nuclei from which the male nucleus has approached. It is thickest opposite the middle of the male nucleus, and exactly recalls the texture of the body-cell protoplasm. My Figs. 18, 19 and 20 should be compared with Coker's Fig. 103. I think it is safe to assume that this cytoplasm was brought in by the male nucleus. In Fig. 20 we have an advanced stage of a more usual type of contact than that shewn in Fig. 19. Five of the cases I have observed resemble Fig. 20, while in one (Fig. 21) the male nucleus had approached the female from one side.

The actual entry of the male nucleus was observed on August 31st; while the cases in which the male and female nuclei were in contact are distributed as follows:—one on August 31st, three on September 3rd, two on September 6th, and one on September 12th. This probably has some connection with the outburst of hot weather which began on August 27th, and continued until the end of the month. I have not come across a single example in which the union of the male and female nuclei was so complete that the two had become indistinguishable.

VII.—The Development of the Proembryo.

I have unfortunately not obtained a preparation shewing the first division of the fusion nucleus, but three ovules collected on September 12th, contain bi-nucleate proembryos. The two nuclei are placed close together at the base of the archegonium, and around and between them is a thin layer of dense protoplasm which may represent in part, at any rate, the cytoplasm of the male cell (Fig. 22). Fig. 23 shews the second division of the fusion nucleus. The small size of the spindle as compared with the resting nucleus which preceded it is apparently a characteristic

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[1] A. A. Lawson. "The Gametophytes, Fertilisation, and Embryo of *Cryptomeria japonica*." Annals of Botany, Vol. XVIII., 1904, p. 417.

feature of the proembryo nuclear divisions of Gymnosperms. In Fig. 23 two nuclei remain in the pollen-tube, while in Fig. 22 there are two nuclei in the upper part of the archegonium which have the appearance of having come from the pollen-tube. It seems not unlikely that in both cases these nuclei have resulted from the division of the second male nucleus. The next stage is the separation of the four daughter nuclei, and the initiation of wall formation between them, (Fig. 24). I have not found anything corresponding to the extensive free cell-formation which Arnoldi[1] has described as occurring in *Cephalotaxus*, and Jäger[2] in *Taxus*. In the four-nucleate stage which I have drawn we see the second male nucleus, which is undivided, remains in the pollen-tube with a vegetative nucleus close to it. I have seen exactly the same state of things in another pollen-tube which was in contact with a multicellular proembryo. In the next stage of which I have a preparation the proembryo consists of seven cells, six of which form one tier, open above, while a single one is placed at the tip (Fig. 25). Further divisions result in the cutting off of rosette cells from the upper tier, and the formation of a cluster of cells at the tip instead of a single one. At first all the tiers are short as indicated in Fig 26 which is a proembryo of September 19th. In Fig. 29 the seeds of this date are shewn as they appear to the naked eye. Fig. 27 represents the condition exactly a month later, when the suspensors have begun to elongate in both directions, apparently demolishing the rosette cells in their upward growth. Fig. 28. is a diagrammatic longitudinal section of a seed at this date.

I can detect no starch in the unfertilised egg, the egg in which the nuclei are fusing, or the bi-nucleate proembryo, but it appears in considerable quantity at the four-nucleate stage. The sudden appearance of the starch is difficult to account for. Plastids must either have been present in the egg or have been brought in in the dense cytoplasm of the sperm cell. At the stage drawn in Fig 25 the tip cell and the lower parts of the cells of the upper tier are packed with starch, while the grains in the upper part of the egg cell are few and scattered. Fig 26 shews a stage in which the entire proembryo, with the exception of the rosette cells is densely filled with starch grains; in the rosette cells comparatively few are to be found. When the suspensor tier has elongated, as in

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[1] W. Arnoldi. Loc. cit.

[2] L. Jäger. Loc. cit.

Fig 27, the starch grains have disappeared from the tip cells and are confined to the suspensors as described by Coker[1] for *Taxodium*.

The number of suspensors is sometimes four and sometimes six.

VIII.—THE NUMBER OF CHROMOSOMES IN THE FEMALE GAMETOPHYTE.

I have made an attempt to count the chromosomes in one of the two cases in which I observed the division of the nucleus of the central cell of the archegonium (Fig. 13). One of the daughter nuclei has six V-shaped chromosomes besides fragments which appear to bring the total number up to eight. In the other daughter nucleus five V-shaped chromosomes could be distinguished and again the cut pieces seemed to make up the number eight. Each daughter nucleus appeared in two successive sections. In one dividing endosperm nucleus which I examined the chromosomes were grouped at the equatorial plate just before their journey to the poles. Here I was able to count fifteen, and I think that it is not unlikely that one was missed owing to foreshortening. I also made out eight chromosomes in three young endosperm nuclei. I am fully aware that, considering the great difficulty of accurate counting, a few observations like these are quite an insufficient basis for a general statement as to the number of chromosomes in the female gametophyte of *Torreya californica*. If further work should confirm the number eight it will be of some interest however, since Strasburger[2] has recently shewn that *Taxus baccata* possesses eight chromosomes in the gametophyte, thus resembling *Ceratozamia mexicana*, and differing from the other Conifers for which the number twelve is characteristic.

IX.—SUMMARY.

The germination of the megaspore takes place about the end of June. Throughout July the endosperm is developing, and at the beginning of August archegonia make their appearance; there are two, three or four in each ovule, three being the most usual number. The neck generally consists of one tier of four or six cells. The division of the central cell was observed in two archegonia from one ovule collected on August 20th. No ventral canal *cell* is formed, and it seems that the nucleus must be very short-lived.

[1] W. C. Coker. Loc. cit.

[2] E. Strasburger. "Anlage des Embryosackes und Prothalliumbildung bei der Eibe." Festschrift zum Siebzigsten Geburtstage von Ernst Haeckel, Jena. Gustav Fischer 1904. Reviewed Bot. Gaz., June, 1904.

The two sperm-nuclei are enclosed in a single cytoplasmic sheath. Only one is functional, but it is not distinguishable in size from the other. Fertilisation occurs early in September, that is between three and four months after pollination. The male cell contributes to the cytoplasmic sheath of the fusion nucleus. Wall formation is initiated when only four nuclei are present, and no trace was found of the extensive free cell-formation which has been described for *Taxus* and *Cephalotaxus* and is regarded as a primitive character.

The number of chromosomes in the female gametophyte appears to be eight.

X.—Conclusion.

I began the study of the spore development and sexual organs of *Torreya californica*, not unprepared to find at any rate some Cycad-like traits, but this expectation has scarcely been realised. If I have counted the chromosomes correctly, the number agrees with *Ceratozamia* (and *Taxus*) and differs from that usually found in Conifers; but in no other point have I succeeded in recognising any additional evidence for the relationship of *Torreya* with the Cycads,—a relationship which is clearly suggested by the structure of the seed and seedling.

EXPLANATION OF PLATES VII., VIII., and IX.

Illustrating A. Robertson's Paper on "The Sexual Organs and Fertilisation in *TORREYA CALIFORNICA*."

nuc., nucellus; *i.*, integument; *e.*, endosperm; *ep.*, "epithelium" of endosperm; *c.*, cuticle; *p.t.*, pollen-tube; *t.n.*, pollen-tube nucleus; *b.c.*, body-cell; *s.c.n*, stalk-cell nucleus; *v.n.*, vegetative nucleus (either stalk or tube nucleus); ♂$_1$ functional male nucleus; ♂$_2$ second male nucleus; *j.c.*, jacket cell of archegonium; *ar.*, central cell of archegonium; ♀ egg-nucleus; *n.c.*, neck cell of archegonium; *pro.*, proembryo; *r.*, rosette-cell; *s*, suspensor; *t.*, tip cell.

§ All the drawings are from material collected this year.

[Where a figure is drawn from more than one microtome section the number of sections from which it is reconstructed is given just before the magnification].

Plate VII.

Fig. 1. Longitudinal section of a bi-nucleate embryo-sac. June 30th. Three sections (× 400).

Fig. 2. Radial longitudinal section of the base of a female prothallus shewing ordinary endosperm cells, poor in contents, and the "epithelial" layer at the base, followed by crushed nucellar tissue. *g.*, a globule of unknown composition, turning yellow with Orange G. September 16th. (× 400).

Fig. 3. "Epithelial" cells from a tangential section of the lower part of the endosperm. September 16th. (× 400).

Fig. 4. Diagram of a longitudinal section of an ovule without its arillus, at the stage when young archegonia are appearing. August 6th. (× 28).

Fig. 5. An archegonium with two neck cells from the section shewn in fig. 4. (× 400).

Figs. 6 and 6A. Successive sections of an archegonium; fig. 6 shewing a neck cell nucleus dividing, and fig. 6A shewing an undivided neck cell. August 12th. (× 400).

Fig. 7. Four-celled neck of an archegonium in transverse section. September 3rd.) (× 400).

Fig. 8. Six-celled neck of an archegonium in transverse section. August 25th. (× 400).

Fig. 9. Six-celled neck of an archegonium in longitudinal section shewing a periclinal wall. August 25th. (× 400).

Fig. 10. Transverse section of a female prothallus with three archegonia cut at the level of the nucleus of the central cell. August 20th. (× 93).

Fig. 11, Longitudinal section of an archegonium with its surrounding jacket cells. August 20th. 3 sections. (× 400).

Fig. 12. Pollen-tube. July 19th. (× 400).

Plate VIII.

Fig. 13. Longitudinal section of upper part of an archegonium shewing four neck cells and the oblique spindle of the division of the central cell. August 20th. (× 400).

Fig. 14. Longitudinal section of upper part of an egg cell shewing the egg nucleus with its chromatin collected into the centre and above it a darkly staining reticulum which may represent the remains of the ventral canal nucleus. September 9th. (× 400).

Fig. 15. End of pollen-tube with body cell behind and stalk and tube nuclei together in front. Slight indications of radiate structure in the protoplasm of the body cell. September 6th. 2 sections. (× 400).

Fig. 16. Body cell with two male nuclei. The nucleus marked marked ♂$_1$ was nearer the neck of an archegonium. September 6th. (× 400).

Fig. 17. End of pollen-tube in contact with the neck of an archegonium. The body cell is elongated so that ♂$_1$ and ♂$_2$ are much separated. One vegetative nucleus is present close to ♂$_2$ September 6th. (× 400).

Fig. 18. Longitudinal section of archegonium into which the front parts of a part of a body-cell with ♂$_1$ has entered, while the rear part of the body-cell with ♂$_2$ remains in the pollen-tube *n*., veg nucleus of p.t. or a neck cell, probably the latter. August 31st. 4 sections. (× 400).

Fig. 19. Longitudinal section of archegonium shewing contact of the male nucleus and egg nucleus. August 31st. (× 400).

Fig. 20. Longitudinal section of archegonium shewing the male nucleus becoming engulphed in the egg nucleus. September 3rd. (× 400).

Fig. 21. Contact of the male nucleus with one side of egg nucleus. September 3rd. (× 400).

Plate IX.

Fig. 22. Longitudinal section of bi-nucleate proembryo. The two nuclei in the upper part of the archegonium may be the products of division of the second male nucleus. September 12th. 3 sections. (× 400).

Fig. 23. Longitudinal section shewing second division of fertilised egg. The two nuclei remaining in the pollen-tube are probably the products of division of the second male nucleus. September 6th. 2 sections. (× 400).

Fig. 24. Longitudinal section of four-nucleate proembryo. The large nucleus left in the pollen-tube is probably the second male nucleus. September 16th. 7 sections. (× 400).

Fig. 25. Longitudinal section of a proembryo with six cells open above and one tip cell. The nucleus in the upper part of the archegonium is probably the second male nucleus, or one of its products of division. September 12th. (× 400.)

Fig. 26. Longitudinal hand section of a proembryo in which the rosette cells, suspensors and tip cells are already organised. cf. Fig. 29. September 19th. (× 400).

Fig. 27. Longitudinal section of a proembryo whose suspensors have begun to elongate. cf. Fig 28. October 19. (× 400).

Fig. 28. Diagrammatic longitudinal section of a seed without its arillus. cf. Fig. 27. October 19th. (× 28).

Fig. 29. Shoot bearing a pair of seeds. cf. fig. 26. September 19th. (Nat. Size).

Fig. 29A. Seed of same date as fig. 29, surrounded by its four bracts. (× 2).

Fig. 29B. Bracts removed, seed with arillus exposed. (× 2).

Fig. 29C. Arillus removed, seed with integument exposed. (× 2.)

STUDIES ON CYANOPHYCEAE.

By F. E. Fritsch, B.Sc., Ph. D., F.L.S.

III.—Some Points in the Reproduction of Anabaena.[1]

[With Plate X.]

INDEPENDENTLY of the reproduction of the Cyanophyceae by means of hormogonia, the sole certain method known to us is that by means of spores and gonidia. Spores are now known in a considerable number of blue-green genera, as will be seen by reference to Brand's recent synopsis of them,[2] but a large number of forms remain, in which they have not yet been found, nor are the details of their formation and germination fully known in those genera, where their occurrence is an established fact. The most important contributions from this point of view are due to Borzi, who in his "Note alla Morfologia e Biologia delle Alghe Ficochromacee,"

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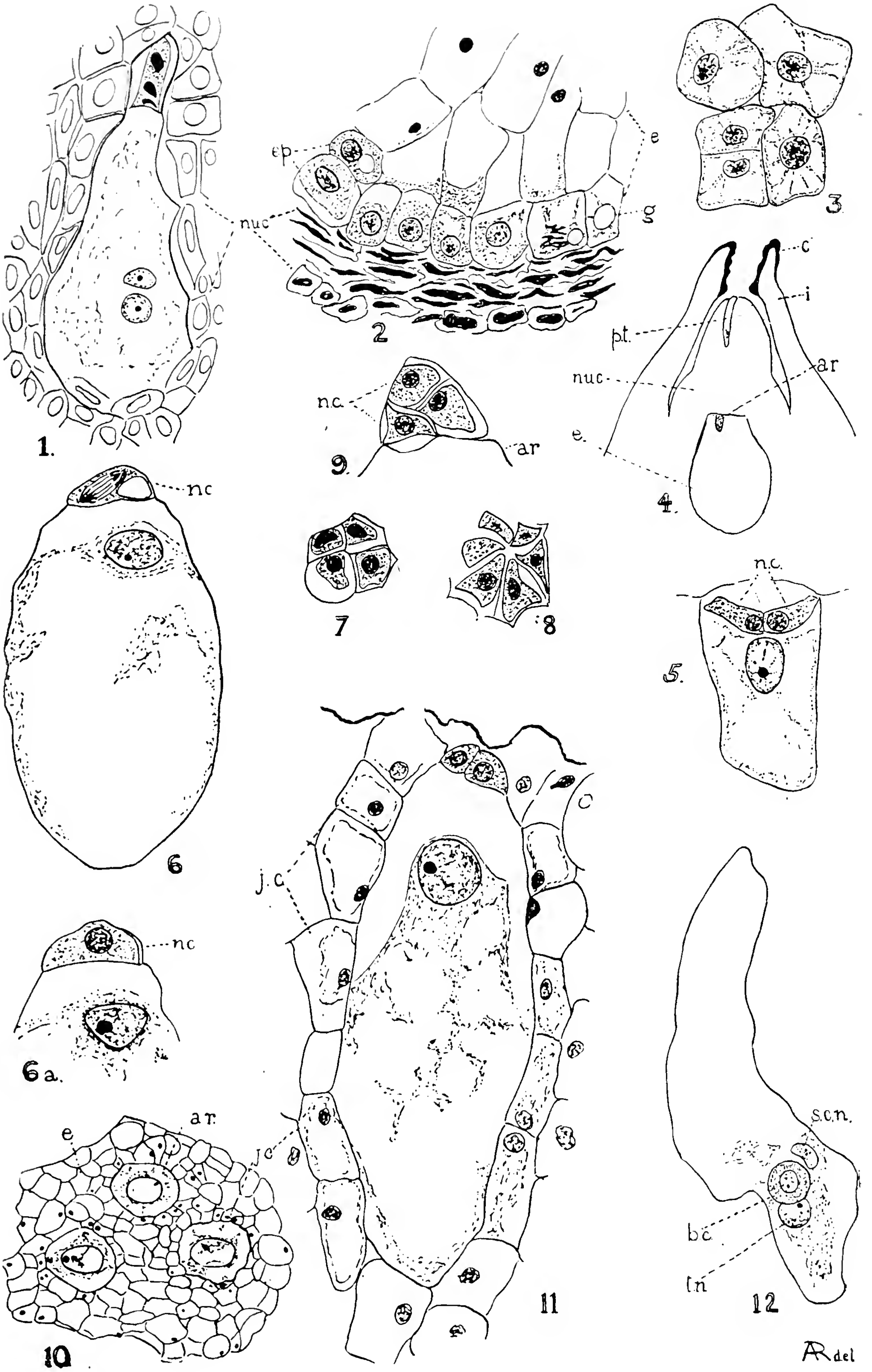
[1] No. 2 of this series, dealing with the structure of the investment and the spore-development of some Cyanophyceae, is about to appear in the Beihefte zum Botanischen Centralblatt (Bd. 18, Abt. I., Heft 2), pp. 30-50 and Tab. VII.

[2] cf. Brand in Beihefte z. Botan. Centralbl., Bd. xv., Heft 1, 1903, pp. 37 and 38.



ROBERTSON.—*TORREYA CALIFORNICA.*

Highley lith & imp.

ROBERTSON _ TORREYA CALIFORNICA.

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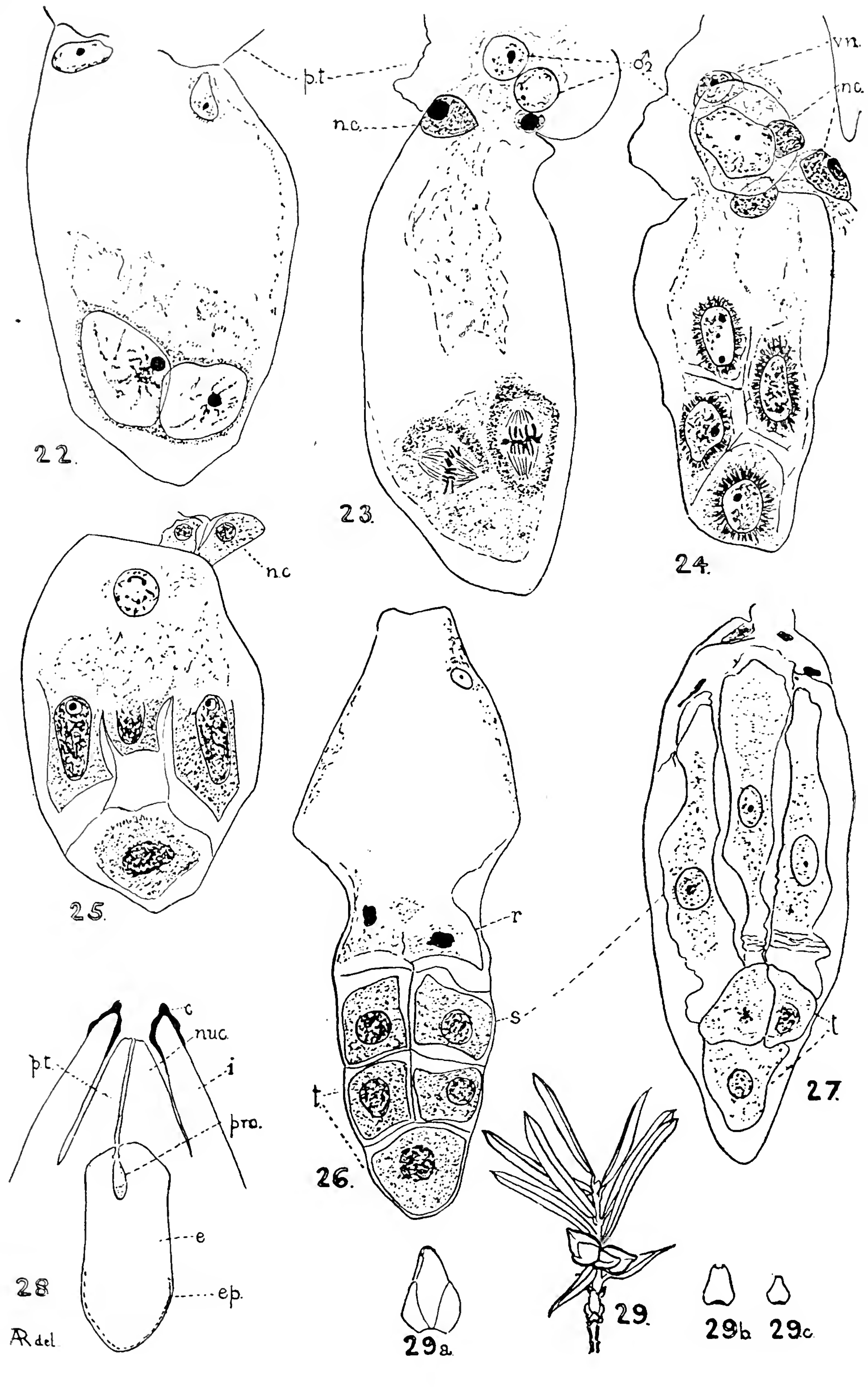

ROBERTSON.—*TORREYA CALIFORNICA.*

published during the years 1878-1882,[1] besides considering the general morphology of the vegetative structure, describes the spores of a number of genera in detail and also gives data concerning their development and ultimate fate. Brand has also done work in this direction and was the first to discover the spores of *Gloeocapsa alpina*, Naeg.[2] and of certain species of *Nostoc (N. commune, N. microscopicum, N. muscorum)*.[3]

The details of the spore-development in *Anabaena* will be described elsewhere[4] and a brief recapitulation will suffice here. The spores were developed very abundantly in the material of *Anabaena Azollae*, Strasburger, which formed the subject of my investigations. Cells about to develop into spores—I have termed them sporogenous cells—exhibit two well-marked integuments at an early stage. The inner one of these (inner integument) completely envelopes the protoplast; it is colourless and apparently of a more or less gelatinous nature. The outer one (cell-sheath), which becomes more and more pronounced as the spore matures, takes the form of a hollow, more or less cylindrical sheath, surrounding the inner investment; it is individual to each cell, the transverse walls of the filament being constituted by the inner investment only. As the sporogenous cells approach maturity they become separated from one another by the formation of intermediate mucilage, while the cell-sheath closes round the open ends of the spore, forming a complete (always ?) outer investment or exospore. The observations, described in the second article of this series, seem to show that the two investments are present at a very early stage and merely attain a more distinct differentiation as the spore matures. However, if either of the two membranes is a newly formed structure it is certainly the exospore (cell-sheath), which is very difficult to distinguish in early stages.

The formation of the spores in the case in question was almost invariably centrifugal, *i.e.* spore-formation commenced in cells distant from the heterocysts and gradually advanced towards them. It is customary to unite certain species, otherwise resembling *Anabaena*, but which have centripetal spore-formation, in the sub-

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[1] Nuovo Giornale Botanico Italiano, Vols. 10, 11 and 14.

[2] cf. Brand, Der Formenkreis [von *Gloeocapsa alpina*, Näg. Bot. Centralbl., Vol. lxxxiii., 1900, p. 228, Figs. 6-9.

[3] Brand. Morphologisch-physiologische Betrachtung über Cyanophyceen. Beihefte z. Botan. Centralbl., Bd. xv., Heft 1, 1903, p. 36.

[4] cf. foot-note 1 on page 216.

genus *Sphaerozyga*.[1] The latter was at one time regarded as a distinct genus, but since it has been shown that both kinds of spore-formation occasionally occur in the same species, its generic position has been abandoned. From what is known, it would appear that the kind of spore-formation,—whether centripetal or centrifugal,—is in some way correlated with external conditions; these must, however, primarily exert an influence on the heterocysts, since it is in relation to these latter, that the alteration in the phenomenon depends. All this suggests, that the heterocyst plays an important part in spore-formation, and a consideration of the genus *Rivularia* will force us to the same conclusion.[2]

Although the mature spores are certainly capable of passing through a long resting period, in the material, which I investigated, germination can evidently take place very soon after their maturation. If filaments containing fully-developed spores are placed under unfavourable conditions (*e.g.* gradual dessication), the vegetative cells become paler and paler and die off, whilst the spores loose the granular appearance of their contents and these latter become homogeneous and highly refractive. Such spores are evidently in the resting condition and are capable of germination, as soon as favourable conditions are restored. These cells are therefore scarcely spores (Dauerzellen) in the physiological sense, which Brand applies to them,[3] according to whom spores are distinguished from gonidia amongst other points by the resting-period, through which they pass, and I shall have occasion in a moment to refer to other features, which show that the morphological and physiological distinction of spore and conidium is not always a sharp one.

The germination of the spores was observed *in situ* in the thread and a very large number of cases was examined. Considerable variation occurs in the mode of germination, but it in all cases leads to the formation of a young thread, possessing all the characters of the *Anabaena;* in one case only, which I have unfortunately omitted to figure, did the contents of the spores seem to have germinated and divided along two planes, somewhat after

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[1] cf. Palla, Beitr. z. Kenntn. des Baues des Cyanophyceen-Protoplasts, Pringsh. Jahrb., Vol. 25, 1893, p. 545; also Schmidle, zur Entwickelung von *Sphaerozyga oscillarioides* (Bory) Kütz. Ber. Deutsch Bot. Gesellsch. Vol. xiv., 1896, p. 393; see also Fritsch, Studies on Cyanophyceae, No. I.—NEW PHYTOLOGIST, Vol. III., No. 4, p. 94.

[2] This will probably form the subject of the next article of this series. I have commented on the possible functions of the heterocysts in relation to spore-development in the first of the studies (see especially p. 93, loc. cit).

[3] Brand, Morpholog.-physiol. Betrachtungen, etc., loc. cit., p. 45.

the manner of a *Chroococcus* or *Gloeocapsa*. This is the only indication I have had of the occurrence of such a stage in the life-cycle of *Anabaena*, such as has been suggested by Hansgirg and others. The fact remains, however, as Brand[1] has also pointed out, that *Gloeocapsa* and other unicellular Cyanophyceae are almost invariably associated with these filamentous forms; this was also the case in the material in question.

In the first type of germination to be described the contents are slowly protruded from the spore, apparently by the excretion of mucilage from the protoplast. The emission of the contents is a very gradual one,—probably taking several hours, if not days and the stages figured are therefore derived from the germination of different spores. Large numbers of cases were observed, representing all intermediate stages, and I think there can be no doubt of the phenomenon taking place as now described. The first indication of the commencing protrusion of the contents is their contraction and the appearance of a small colourless papilla at one end of the spore (Fig. 22); apparently this is in direct continuity with the protoplasmic contents, still enclosed in the membranes of the spore. This papilla (cf. p. 37 of the second article of the series) apparently pushes its way through the terminal portion of the exospore (which has perhaps in these cases not formed fully around this end of the spore ?), which surrounds it like a sheath; the colourless papilla itself is probably the terminal portion of the inner investment, which is pushed out in advance of the escaping protoplast and probably serves to protect it at first. The papilla is gradually followed by the protoplasmic contents, which leave a space behind them, occupied by colourless mucilage (cf. Figs. 21, 23, 24, 25 and 26). The aperture at the end of the spore, through which the contents pass out is often so narrow that they are distinctly compressed at this point, acquiring a biscuit-like shape (Figs. 20 and 25), and recalling the shape of the zoospore of a *Vaucheria* during its escape from the zoosporanginm. Numerous stages with the contents half liberated in this manner were observed (Figs. 23, 24 and 25), and formed an illustrative transition to cases in which only a small portion of the contents still remained inside the spore (Fig. 26). Their entire liberation is followed by the escape of the mucilage, to which it is due, and this is then generally visible surrounding the liberated spore-contents as a thin, transparent envelope of a spherical shape,[1] and of about three times the diameter of the spore (Fig. 15)

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[1] Loc. cit., p. 46.

whilst the empty case of the latter (with exospore and endospore intact) lies at the side and plainly exhibits the now wide aperture, through which the contents were protruded; for in the large majority of cases the pressure of the mucilage in the spore is too considerable and the originally narrow terminal aperture gets more or less widened out, as shown in Figs. 15 and 26. The empty spore then has a cup-shaped form with the open edges often more or less turned back (cf. Fig. 26). Although the mucilage, which causes the liberation of the spore-contents is mostly readily visible as a surrounding envelope after their escape, it is very difficult to demonstrate it during the actual process of protrusion. Staining with Vesuvin for instance results in the first place in the external mucilage taking on a deep brown colour and this obscures everything else. In isolated spores, however, it seemed as though the mucilage inside the spores with half-liberated contents were coloured faint brown by the stain. Iodine, as is to expected, does not affect it. The giving way of the spore-membranes to the pressure of the mucilage may take place at an earlier or later stage in the liberation of the contents (cf. Fig. 20 with Fig. 26). In some few cases the contents of the spore had been liberated laterally instead of terminally (Figs. 10 and 21); this is probably due to the spores lying closely together, instead of considerably separated, as is usual, so that the pressure of the mucilage inside the spore forces open a lateral aperture. The liberated contents are in most cases without a distinctly differentiated membrane at first. Abundant formation of mucilage inside the spore may lead to still another (rarely occurring) variation of this type of germination, in which a part of the spore-membrane is split off as a whole and thus forms the necessary opening for the liberation of the contents (cf. Figs. 3, 7 and 10). This lid is either pushed out in front of the exuding mucilage (Figs. 3 and 7), or may remain attached on one side to the rest of the spore-wall (Fig. 10). The detached portion may be either terminal (Fig. 10 at *t*) or lateral (Fig. 10 at *l*).[2] In this type of germination it frequently happens that the contents of the spore are not liberated at all, but germinate *in situ*, the filament formed by the division of the spore-contents gradually pushing its way out of the open spore-membrane (cf. Fig. 7). But as a rule in all the cases as yet described, the spore-contents do

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[1] Fig. 16 represents an interesting case in which the contents of two spores have been liberated towards one another and are enveloped in a common mucilaginous envelope, due to the confluence of the mucilage of each.

[2] In Figs. 3 and 7, the detached portion has been lost in the surrounding water.

not undergo division until fully liberated, but this is not invariably the case.

In the second type of germination the exospore itself becomes mucilaginous—possibly the endospore also—and gradually swells up, its outer circumference increasing more and more in extent; in this way the contents of the spore, whilst retaining their original position in the thread, come to be surrounded by a thick zone of colourless mucilage, which may be stratified or not (Figs. 1, 2, 9, 13 and 14). Frequently when several spores, lying side by side behave in this way the enveloping mucilage becomes more or less confluent (Figs. 2, 11 and lower part of 13). The contents of the spore may begin to divide very soon after the transformation of the exospore commences (cf. Figs. 1, 11 and 13), although just as frequently the division of the contents is postponed until the mucilaginous envelope has attained considerable dimensions (Fig. 4). In this type of germination a well-marked wall generally becomes apparent round the contents at an early stage. In many cases the whole wall of the spore does not become mucilaginous, but some portions of it remain unaltered (cf. Figs. 5, 6 and 8); we may regard this as a combination of the two main types of germination. Such cases often resemble those in which the membrane is widely opened by the escaping mucilage in the first type of germination. On the whole the first type of germination and its variations are more frequently met with.

The germination of the contents of the spores may (in both of the above types) take place in various directions, most commonly in the direction of the axis of the filament (Figs. 1, 2, 7, 9 and 13); not rarely, however, the axis of the new filament is perpendicular (Figs. 14 and 10 at *l*), or oblique (Fig. 8) to that of the mother filament. This appears to depend on the mode of opening of the spores and indirectly on those influences, which lead to this latter phenomenon (cf. p. 220). Heterocysts began to develop in the young chains at a very early stage, a 3-celled filament not uncommonly having one (rarely two) of its cells transformed into these structures (cf. Figs. 7, 8 and 9); the young plant thus even at this stage manifests the tendency towards the production of heterocysts, so prevalent in the mature filaments (cf. Fritsch, loc. cit. p. 89). The extreme case is attained, when the contents of the spore on liberation develop into a single heterocyst,—a very rare phenomenon, which appeared to be the result of germination of dwarf-spores with very few contents. These points again suggest a number of problems with reference to the heterocyst, which I do not feel prepared to dwell on here, but hope to return to at some future date. These cases are probably to be looked upon as abnormal, but that does not diminish their interest.

The mature spores are not always elongated in the direction of the axis of the filament ; in some cases, though not very commonly, their long axis is placed more or less transversely or obliquely to that of the filament (cf. Figs. 17, 18 and 19). The germination of such chains of spores often gives rise to complex appearances, such as that shown in Figs. 17 and 18.

Borzi describes the germination of the spores in *Anabaena* in the following words :[1] "The first indication of germination is the appearance of a very delicate diaphragm in the interior of the spore, by means of which the contents appear to be divided into two cells. They are the initial elements of the new thread. Afterwards the membrane breaks on one side and the germ unfolds freely outside of the spore. Then the young thread appears, wrapped up in a thick layer of quite colourless mucilage." Borzi does not figure these stages in *Anabaena*, but in his Figs 7-10 on Plate X. the germination of spores of *Sphaerozyga* is shown. The lower drawing in fig. 7 represents a spore, whose contents have undergone division to form a filament of five cells (cf. my Figs. 9 and 12), whilst in the upper figure one end of the filament is beginning to push its way out of the spore (comparable to my Fig. 7). This type of germination belongs to the first of the two above described, but as I have already emphasised above in the material, I examined, division did not as a rule take place previous to the emission of the contents ; however this is a feature, which is likely to vary considerably and does not seem of much importance. Borzi either did not meet with the different types of gemination I have described, or overlooked them.

The germination of the spores of *Nostoc*, which is unlikely to differ essentially from that of *Anabaena*, is shown on plates VII. and VIII. and here (Plate VII., fig 9, Plate VIII., Fig. 10) the spore-contents are not divided when first liberated. It may be well to quote Borzi's description of the germination in this genus. The first indication of germination " is the rupture of the membrane of the spore at some point of its surface. This is probably due to the sudden metamorphosis of the endospore into a

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[1] Borzi. Note alla morfologia e biologia delle alghe Ficochromacee, Nuov, Giorn. Bot. Ital. Vol 10. 1878. p. 261.

mucilaginous substance and to its rapid swelling under the action of the water.........The contents represent the initial cell of the new filament......In the interior of the protoplasm, which is homogeneous throughout, no trace of a transverse division is to be distinguished up till now. The surrounding layer of mucilage continuing to thicken, the initial element of the new thread is step by step liberated from the envelope of the spore ; then after becoming a little elongated, it divides in the transverse direction. The two new cells, thus formed, continue to divide indefiinitely in the same manner and always in the same direction (p. 255)." According to Borzi the endospore during germination thus becomes transformed into a delicate layer of mucilage, which envelopes the initial cells of the new thread (see also loc. cit. p. 254). I am however, rather inclined to imagine that the mucilage which causes the liberation of the spore-contents, is not (at least, not entirely) derived from the endospore, since both exospore and endospore are still recognisable in the spore-membrane after the contents are liberated (cf. Figs. 10 and 15). On p. 257 (loc. cit.) Borzi describes the somewhat different germination of *Nostoc lacustre* ; here " the initial cell of the young filament is divided a good deal before the membrane of the spore breaks open ; in the interior of the spore the division of the cell into two new elements takes place. Soon after the envelope of the spore breaks on one side......and the two cells, becoming liberated appear enveloped by a thin sheath of transparent mucilage." Borzi regards this method of germination, which differs from the other in the primary division taking place earlier, as normal in *N. lacustre*, which seems plausible. In one of the spores of fig. 8 on Plate VIII. a detached piece of the endospore is seen on one side, recalling one of the types of germination, I have described above ; in the other figures however the detached piece is not figured.

In his recent treatise on Cyanophyceae Brand devotes a section to the discussion of the gonidia ; these reproductive cells have long been known in the Chamaesiphoneae, where they are very well differentiated, and the older literature also contains a number of observations, pointing to their occurrence in other members of the blue-green Algæ. Brand himself carefully describes their occurrence in *Phormidium uncinatum*, Gom.,[1] where they arise singly by rejuvenescence of the whole or of the greater part of the cell-contents ; exceptionally several gonidia (microgonidia)

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[1] Brand. Morphol.-physiol. Betrachtungen, etc. Loc. cit. pp. 46 and 47.

are formed in a cell. The same author has also observed the liberation of gonidia from the heterocysts of *Nostoc commune*[1] and *N. microscopicum*,[2] and subsequently adds that similar structures also develop from ordinary vegetative cells in these species;[3] they are stated to differ in no way, from those derived from the heterocysts, but Brand was unable to observe their mode of origin, and bases his conclusions on the fact, that he met with short rows of gonidia, which were in direct contact with ordinary vegetative cells on each side.[4] He concludes from analogy however that, as in other cases, they are formed by rejuvenescence and are liberated by the membrane of the original cell being cast off.

In some material of *Anabaena*. which had been separated from the rest and not examined for some months, processes, recalling gonidia-formation, as described by Brand, had set in. The cells of the filaments differed in shape from the normal one; they were generally more or less spherical with somewhat flattened ends, and differed from the usual elongate-elliptical shape (Fig. 29). Numerous heterocysts were however present, so that this appears to be a constant feature of the species. In some of the filaments the cells midway between two heterocysts were slightly larger than those immediately adjacent to the latter (cf. Fig 29), but there were no true indications of typical spores. The detailed structure of the investment, as I have described it elsewhere and briefly above, was remarkably clear, and it seems possible that some of the cells were in a sporogenous condition; however, the cells were arranged in coherent rows and were never separated from one another as is the case in mature spores; nor did the cell-sheath in most cases seem to have extended round the ends of the mother-cells of the gonidia. In many of these cells (both in the enlarged and the ordinary ones) the contents were markedly rounded off and contracted, and could in most cases be seen to have acquired a new membrane of their own (frequently exhibiting two complete investments) inside that of the mother-cell (Figs. 27 and 29). Large numbers of cases were found in which the cell-membrane had

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[1] Brand, Bemerkungen üb. Grenzzellen u. üb. spontan rothe Inhaltskörper d. Cyanophyceen. Ber. Deut. Bot. Ges. Vol. 19. 1901. p. 154, et seq.

[2] Brand, Morphol.-physiol. Betrachtungen, etc. Loc. cit. p. 48.

[3] Loc. cit., p. 49.

[4] I do not quite gather what were Brand's actual reasons for regarding these cells as gonidia, and it seems just possible that they may be the results of spore-germination according to the second of the above types.

opened laterally (very rarely terminally) and had protuded the contents as a spherical gonidium with a well-marked wall, which were then found lying on one side of the empty mother-cell[1] (Figs. 27 and 28). Frequently the wall of the latter becomes distinctly mucilaginous (Fig. 29) and, in these cases, the contents are probably not protuded at all. The contents were never observed to have divided before liberation, and even in the liberated ones this was a rare phenomenon. I think it probable that the liberated gonidium is capable of passing through a resting-period.

If we carefully compare the gonidia-formation with the spore-formation of *Anabaena* we find one marked point of difference; the gonidia are surrounded by a definite wall at an early stage. Brand's Fig. 15 (loc. cit.) of *Phormidium uncinatum* also shows this point (cf. also his Fig. 17), although the fact is not directly stated in the text. In the case described by Brand, the gonidia are quite evidently formed by a contraction (and rounding off) of the cell-contents and in *Anabaena* there is also some contraction, although it is not so marked as in *Phormidium*. On the other hand, the way in which the gonidia of *Anabaena* are liberated quite recalls the liberation of the contents of the spores, the membrane, enclosing the liberated cell, being either broken open on one side or becoming entirely mucilaginous. The main difference therefore lies in the fact that the gonidium has a well-marked wall at an early stage, whereas in the case of the spores this is as a rule not recognisable until some time after the liberation of the contents. Brand summarises the differences between spore and gonidium, as follows:—"Sowohl inbezug auf die Entstehung als auf die schliessliche Entwicklung wäre ein vollständiger Gegensatz zwischen Dauerzellen and Gonidien gegeben, indem erstere ohne Zellverjüngung entstehen und erst nach einer Ruhezeit bei der Keimung Vollzellbildung oder vielleicht auch Vielzellbildung eingehen, während die uns hier beschäftigenden Gonidien durch Zellverjüngung entstehen, dann aber direkt in den vegetativen Zustand übergehen" (loc. cit. p. 45). The rejuvenescence of the contents is certainly much better marked in the gonidia than in the spores, but I am very doubtful about the physiological distinction. I have already pointed out above that the spores seem capable of germination on maturity and the observations I have made on the liberated gonidia point to their not as a rule germinating immediately on liberation. In fact the gonidia-formation in *Anabaena* seemed to

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[1] No case of gradual protusion of the gonidium was observed, comparable to that of the spores, described on p. 219.

me a result of arrested spore-formation, in which owing to some unknown conditions the sporogenous cells ceased to increase in size, whilst their contents contracted slightly away from the cell-membrane and became enveloped by a new (and generally quite thick) wall of their own. The conditions under which these gonidia developed were certainly not very favourable, for the material merely had a thin layer of water, covering the base of the containing glass vessel, to exist on; most of the cells however looked quite healthy. This may possibly indicate the way in which gonidia-formation resulted from spore-formation, *i.e.* as an arrested stage in the development of the latter. In some of the other forms (*e.g.* Brand's *Phormidium*) the power of spore-formation has become entirely lost, which may possibly be a result of the development of a coherent cell-sheath or may be due to other conditions.

In concluding it may be as well to briefly summarise the essential points in the present paper:—

(i.) The spores of *Anabaena* exhibit two main types of germination, in which the contents are either protruded from the ruptured spore-membrane by the formation of mucilage (probably) from the protoplast; or, in which the spore-membrane itself becomes mucilaginous, whilst the contents retain their original position in the thread.

(ii.) The gonidia of *Anabaena* are formed by rejuvenescence and are surrounded by a well-marked membrane (of two layers) before liberation. Their liberation takes place by two methods, recalling those of spore-germination.

(iii.) There is evidence to show that the spores can germinate immediately on maturity, whilst the liberated gonidia seem to pass through a resting period.

University College, London.
November 26th, 1904.

DESCRIPTION OF FIGURES ON PLATE VII.,

ILLUSTRATING F. E. FRITSCH'S PAPER, "STUDIES ON CYANOPHYCEAE."

[All the figures refer to *Anabaena Azollæ*, Strasburger, and were prepared with an Abbé drawing apparatus. Magnifications:—Fig. 1 × 640; Figs. 2 to 9, 11 to 14, 20, 26, × 680; Figs. 10, 15, 25, × 850; Figs. 16, 19, 21 to 24, 27 to 29, × 1500; Figs. 17, 18, × 600].

Fig. 1. Small portion of a filament, showing four spores in the lowest of which the spore-membrane is becoming mucilaginous and the contents have

divided. The cells of the lower portion of the filament are still in the vegetative condition.

Fig. 2. A chain of germinating spores, the membranes of the median ones have become mucilaginous and are partly confluent.

Fig. 3. Lateral liberation of the contents of the spore by detachment of a lateral piece of spore-membrane which had become lost in the surrounding water ; the stratified mucilage, which causes the liberation of the contents, is well seen.

Fig. 3A. The same process, but in this case the liberated contents have already undergone sub-division ; they are surrounded by a well-marked mucilage-envelope.

Fig. 4. The second type of germination ; the spore-membrane has swollen up and the contents lie in the resulting mucilage.

Fig. 5. Part of the spore-membrane only has become mucilaginous ; the young filament is 3-celled, the cell at one end being a heterocyst.

Fig. 6. Here only the central part of the spore-membrane has become mucilaginous ; the contents of the spore have undergone sub division.

Fig. 7. Filament with germinating spores, two of which have opened by the detachment of a terminal piece of the membrane (lost in the surrounding water) and have each given rise to a 6-celled filament, whose base is still enclosed in the cup-shaped remnant of the spore-membrane; one of the lower spores is germinating according to the second type.

Fig. 8. Compare description of Fig. 5.

Fig. 9. A germinated spore, whose membrane has become mucilaginous, and encloses a young 4-celled filament.

Fig. 10. Portion of a filament, illustrating a number of variations of the first type of germination ; *a* and *b* exhibit stages in the ordinary protrusion of the contents ; *t* shows the liberation of the contents by the detachment of a terminal piece of the spore-membrane, which is turned on one side like a lid ; *l* shows a similar case, but here the lid is lateral.

Fig. 11. Germination of a chain of spores according to the second type ; the mucilage round the dividing contents is partly confluent.

Fig. 12. Compare description of Fig. 9.

Fig. 13. Various stages of germination according to the second type. In the lower spores the mucilage round the contents of several spores is confluent.

Fig. 14. Compare description of Fig. 4.

Fig. 15. Germination of a spore according to the first type ; the spore membrane has been forced widely open by the mucilage, which now envelopes the sub divided contents.

Fig. 16. cf. description in text, p. 220, footnote 1.

Fig. 17. Irregular mass of cells, produced by the germination of transversely or obliquely elongated spores (cf. text, p. 222).

Fig. 18. Illustrates the same as Fig. 17 in a somewhat earlier stage.

Fig. 19. A row of four spores, which are elongated in the transverse direction.

Fig. 20. Liberation of the spore-contents according to the first type of germination ; the aperture, through which they are protruded, is so narrow, that they are distinctly compressed at that point.

Fig. 21. Three spores in the mature condition ; the upper one exhibits a slight contraction of the contents previous to their liberation ; the lower one has already partly liberated its protoplast laterally.

Fig. 22. Commencement of the protrusion of the contents in the first type of spore-germination ; a small papilla is visible at one end.

Figs. 23, 24. 25 and 26. Various more advanced stages in the same process (cf. text p. 219).

Fig 27. Formation and liberation of gonidia. In three of the cells the enveloping membrane has become mucilaginous, in one the contents have escaped by a lateral opening from the unaltered membrane of the mother cell. Note the well marked wall round the gonidium.

Fig. 28. Two cells of a filament of four, from which the gonidia have escaped.

Fig. 29 Chain of cells developing gonidia; the membranes of the mother-cells have all become mucilaginous.

METHODS OF SURVEYING VEGETATION ON A LARGE SCALE.

By F. W. Oliver

AND

A. G. Tansley.

[With Plate XI. and Figs. 77-80].

THE object of the present paper is to describe two convenient related methods of surveying and mapping the vegetation of a small area on a large scale. They are only applicable where the ground is fairly flat or gently undulating, and they are essentially intended to exhibit the features of an area in which the vegetation has a distribution which is complex, but definitely related to the variation of physical features within short distances, such as variations of soil, surface-level, slope, water-level and the like.[1]

The methods, both of which we have recently used with success in the survey of a circumscribed salt-marsh occupying the floor of an estuary called the Bouche d'Erquy on the north coast of Brittany, may be termed the "Method of Squares" and the "Gridiron Method" respectively. The former is suitable for the purpose of constructing a general map of an area comprising a considerable number of acres, on a scale of $\frac{1}{250}$ to $\frac{1}{500}$ or thereabouts, the latter

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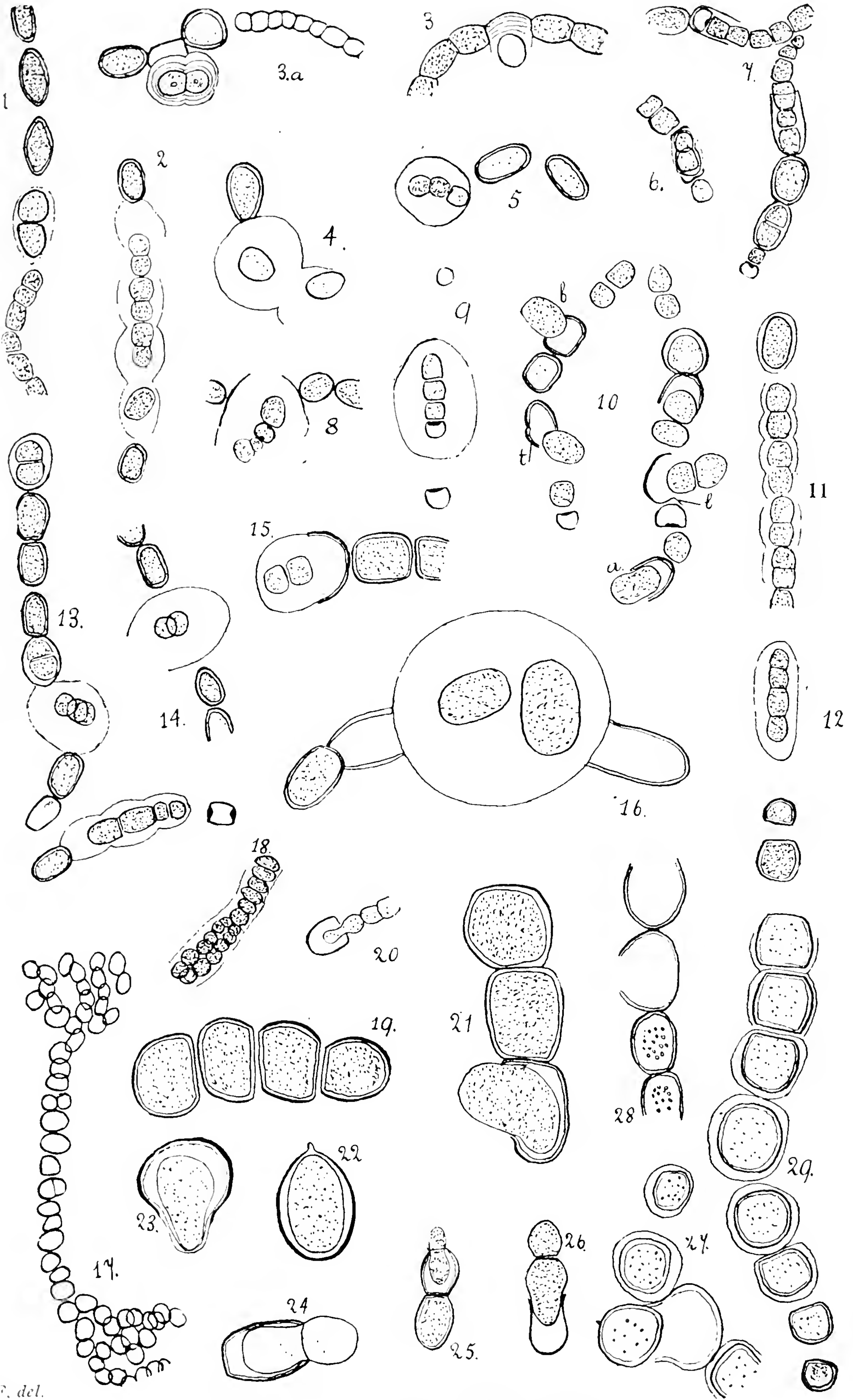
[1] The "Quadrat method" used by the Nebraska School of Ecologists for estimating the number of individuals of different species occurring on a small area (5 metres square) appears to have little in common with our method, though the quadrat maps are in some respects comparable with our "gridirons."



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for very detailed work restricted to small parcels of ground and giving results correct to 6 inches or even less.

THE METHOD OF SQUARES.

The basis of this method is the fact that a square pegged out on the ground can, with a little practice, be quickly and accurately mapped upon ruled sectional paper. A small square is of course easier to map than a large square, but it is well to avoid choosing too small a unit if the survey is to make reasonable progress. We found in the work referred to that a square with a side of 100 feet held the balance pretty evenly between accuracy and dispatch and consequently adopted it as the unit.

To cover the ground to be surveyed with a continuous system of 100-foot squares, the following procedure is convenient A suitable base line (= line α) is selected as in the case of an ordinary land survey. It is chained and posts planted every 100 feet. The starting point of the base line is known as station 0, the 800-foot point as station 8, and so on. From certain selected stations on the base line perpendiculars are ranged with a theodolite on either side. These are chained and posts planted at 100-ft. intervals. The stations on the perpendiculars may be conveniently known as *a*, *b*, *c*, &c., in the direction *above* the base line, and *z*, *y*, *x*, &c., in the direction *below* the base line. In the diagram (Fig. 77) perpendiculars have been raised at stations 5, 13 and 21 and are termed the lines 5, 13 and 21. On the other hand parallels to the base line ranged through *aa*, *bb*, &c., are distin-

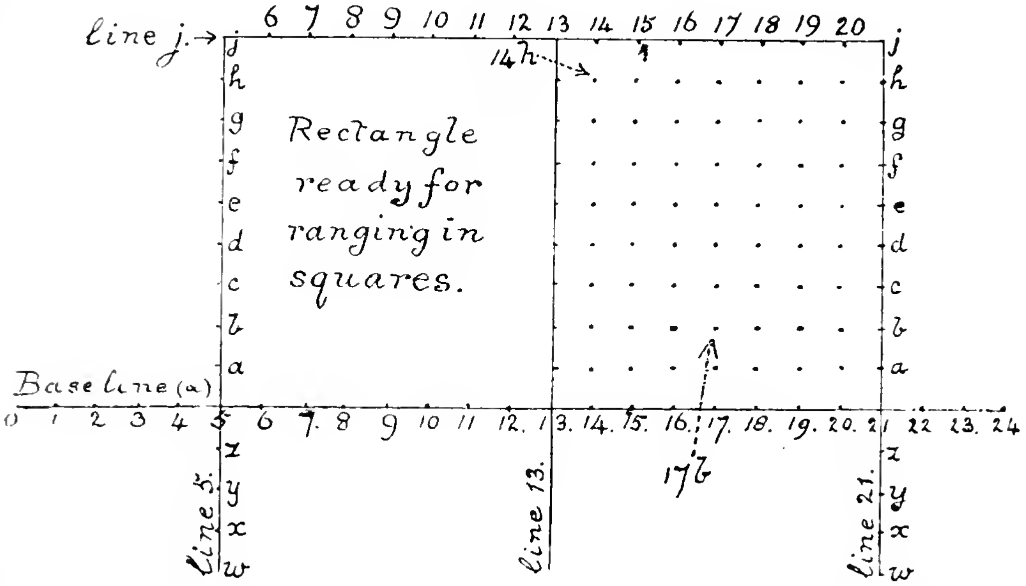


Fig. 77. Illustrates the various operations required to cover an area with a system of squares having 100-foot sides (see text).

guished as the line *a*, the line *b*, etc. For convenience in completing the operation of squaring, the posts on the base line stations and those on the measured perpendiculars should respectively bear numbered and lettered cards conspicuously displayed with their surfaces parallel to the lines on which they are, so that they can be easily seen from the directions perpendicular to these lines.

The rectangles between the perpendiculars are now completed by ranging a parallel, as at *j* (Fig. 77), and posts with numbered cards are planted at 100-foot intervals in the usual way. The final plotting of a rectangle into squares can now be carried out very rapidly by three persons, of whom one plants the corner posts whilst the other two act as linesmen on two adjacent sides of the rectangle, ranging the post to be planted against the corresponding posts on the other two sides. In Fig. 77 two rectangles are shewn, one of which has been plotted into squares.

In actual practice the perpendiculars should be raised at intervals not exceeding 600 feet, whilst the employment of field-glasses is of great assistance in promoting the operation of plotting the ultimate squares.

The posts which mark the corners of the squares are known by the designations of the lines whose intersections they occupy, e.g. 14h, 17b, (see fig. 77). As soon as the squares in a rectangle have been plotted out, the posts should be marked with their designations. Observance of this precaution will save much loss of time, worry and confusion throughout the survey, and its importance cannot be exaggerated.

When the ranging of the squares is complete the existence of any inaccuracy will be manifest. If the posts of the verticals, parallels and diagonals, respectively, stand in proper alignment everywhere the preliminary work of the survey may be accepted with confidence. And it may be counted as a further merit of the system that if posts are removed by mischievous persons it is of little moment, for they can be replaced as a rule by simple ranging. It is prudent, however, to ensure the permanence of the base line by cutting out pieces of turf at each of the 100-foot stations.

Mapping the Squares.

For the purpose of mapping, the rectangles are allotted to small parties of two or three members, one of whom acts as Surveyor. The latter is provided with a field-book consisting of leaves of squared paper, and each 100-foot square is mapped on a single leaf. The form of squared paper which we have found useful in this connection is ruled down to tenths of an inch, whilst every fifth line is slightly, and every tenth line strongly emphasized by deeper printing. A 5-inch square is ruled off in the book for the mapping of a 100-foot square, giving a scale of $\frac{1}{240}$. The physical features are put in first and then the boundaries of the different plant-associations, those which are to be recognised having been previously agreed upon and designated by symbols. Any point within the square can be at once identified in the map by means of imaginary perpendiculars dropped from known points on the sides, so that the surveyor, by standing at the point he wishes to mark, can get two assistants to fix his position by optical squaring from the side lines. In practice, by placing sticks at intervals of 20 feet along the side lines before he begins to map, the surveyor can soon judge his position in the square with sufficient accuracy, without resorting to the use of the optical square. After salient points are marked, the actual boundaries can be rapidly drawn by eye with considerable accuracy. Meanwhile detailed notes are made on the vegetation included in the square, and representative plants are collected, labelled, and pressed.

In this way a map of the whole area is constructed in sections on a scale of $\frac{1}{240}$ and can be dealt with as required. The fact that the data surveyed at once take shape as a map in the field is an attractive feature in the method of squares, since it eliminates the operation (necessary in the method of an ordinary chain-survey) of plotting field-book measurements at home. The automatic test of accuracy given by the fact that errors are at once evident to the eye in the ranging of the corner posts has already been mentioned and is another advantage. Finally the confusion which results in the taking of "off-sets" from ordinary survey lines where complicated features have to be mapped, is entirely avoided by this method of self-contained, but connected, units of area,

To secure a uniform notation of the squares the left-hand bottom corner-post may conveniently give the designation to the square. That is to say, in the case of squares *above* the base line of the two corner-posts on the perpendicular which lies towards the zero-point of the base line, the one nearer the base line denominates the square; in the case of squares *below* the base line it will be the one remote from the base line. In mapping, the denominating or index-post of the square should occupy the left-hand bottom corner in the field-book. By the adoption of this practice the mapped squares will be uniformly orientated and liability to confusion avoided, and at the same time the position of any square on the area in process of survey will be evident. When a square has been mapped it is well to attach a conspicuous band to its denominating post.

A surveyed square is reproduced in the accompanying figure 78. The ruled lines in the field book are omitted.

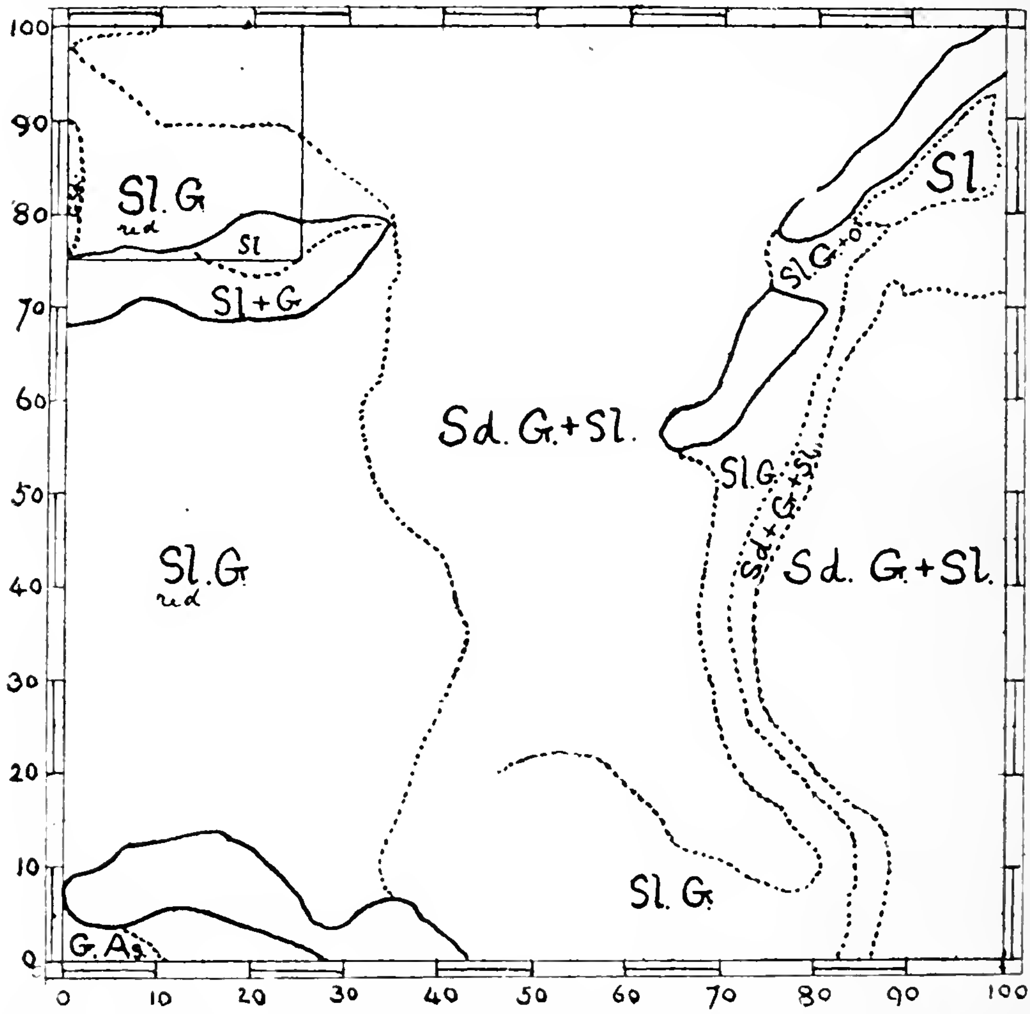


Fig. 78. Map of a square with 100-foot side on a scale of $\frac{1}{240}$. The small portion ruled off at the top left-hand corner is represented on a large scale by the gridiron method in fig. 80. The boundaries of the plant-associations are represented by dotted, the physical features by continuous lines. The scale round the edge of the map is marked in lengths of 10 feet.

This square is a piece of the salt-marsh of the Bouche d' Erquy, already referred to, and although without special value detached from the map of which it is a fragment, it may serve to illustrate the method.

The physical features surveyed are marked in continuous lines, the boundaries of the plant-associations in dotted lines. The former include abrupt depressions or "pans" in the upper right hand, and in the bottom left hand corner of the square. Of vegetation-covering, the right and left hand portions bear the *Salicornia herbacea-Glyceria maritima* association (Sl. G.), whilst on the middle parts grows a turf of *Suæda maritima* and *Glyceria* with a scattering of *Salicornia* (Sd. G. + Sl.) The central Sd. G. is bordered on the right by a narrow belt of Sl. G. and again between this and the Sd. G. of the extreme right there is a second narrow belt in which *Suæda* is dominant in association with *Glyceria* and *Salicornia* (Sd. + G + Sl.)

The notes which accompany this map shew that associations in which *Suæda* is a dominant or a co-dominant lie at a slightly higher level than than those in which *Salicornia* plays a corresponding rôle. Were we in a position to add some dozen spot-levels to this map the relation of the plant-associations to differences in level would be evident. This important feature, however, is fully illustrated in the case of the "gridiron" described below.

THE GRIDIRON METHOD.

The "Gridiron" method is essentially an adaptation of the "method of squares" to a survey of vegetation in greater detail. It is suitable for use where the physical features, with which the different plant formations are correlated, exhibit definite variation within quite short distances. The scale used in the present instance was $\frac{1}{60}$, a square "gridiron" having a side of 25 feet being plotted on a square of paper having a side of 5 inches, so that the tenth of an inch rulings on the paper used (see above) correspond to distances of 6 inches on the ground. This scale allows the insertion of as much detail as is ever likely to be required in mapping vegetation; it is quite easy, for instance, to put in the positions by means of symbols of individual scattered plants of species not forming a constituent of a continuous plant-carpet (e.g. *Obione portulacoides* and *Salicornia radicans* in the specimens given below, Fig. 80).

The method of procedure is as follows. The 25-foot square is first pegged out, and tapes are laid round its sides. Four 25-foot tapes are then laid across the square at intervals of 5-feet, so as to divide the square into 5 strips, each measuring 25 × 5 feet. Thus the "gridiron" is formed (Fig. 79). The tapes corresponding to the bars of the gridiron should run *across* the greatest number of physical features and association boundaries to be mapped, rather than parallel to them. The "bars" of the gridiron of course enable the surveyor to map with greater *relative* accuracy than in the method of squares, where there are no bars, and this is highly desirable in cases where such detailed work is worth undertaking at all. A gridiron can easily be mapped by one person.

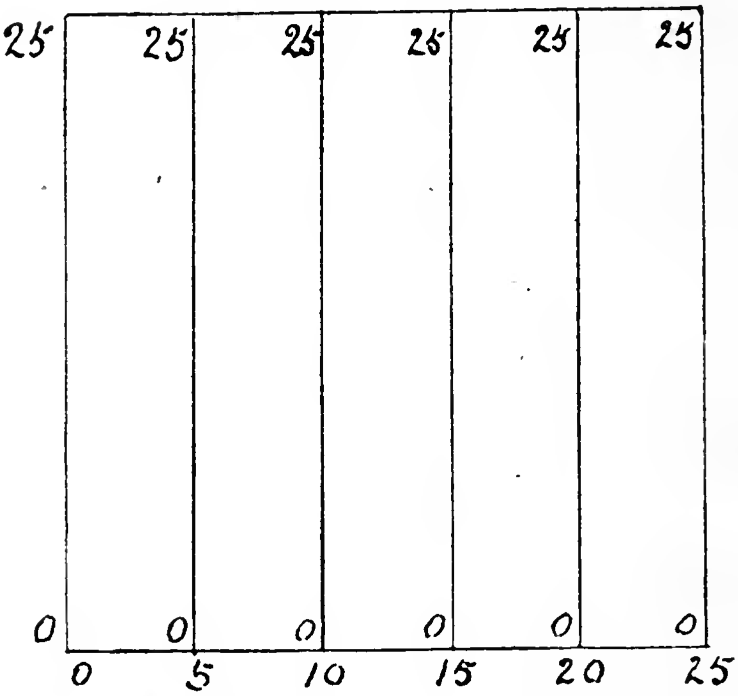


Fig. 79. Mode of laying out the tapes in the Gridiron.

The specimen gridiron shewn in Fig. 80 forms the upper left-hand corner of the square shewn in Fig. 78. The cliffs forming the edges of channels are put in as continuous lines, the boundaries of associations as dotted lines, while the contour lines, one-tenth of a foot apart, are represented by lines composed of alternate dots and dashes. The surfaces of the "pans" and channels, where they are of bare mud or are covered only with algæ (*Rhizoclonium*, *Lyngbya*, *Chroococcus*), are in black. The association symbols used are the same as in the square (see above). The "spot" levels were taken with the theodolite and levelling staff, reading to $\frac{1}{100}$ of a foot, and were corrected to a *datum* (the normal water level at low tide at a certain spot in the channel of the main stream). They represent hundredths of a foot above this *datum*, e.g. 375 means 3.75 feet above *datum*. The contour lines are necessarily approximately correct only; a very much greater number of readings would be necessary to obtain complete accuracy. They have been drawn partly with the assistance of the association boundaries. This course is justifiable in view of the fact that in other gridirons, where the readings are more numerous in the immediate neighbourhood of the association boundaries, the contours drawn from the spot-level data alone frequently follow the boundaries very closely.

A comparison of Figs. 78 and 80 exhibits very well the greater detail which can be shewn on a grid as compared with a square. The enclosed area corresponding with Fig. 80. in the upper left hand corner of Fig. 78 shews, running across its lower part, a continuous line representing the cliff of a channel, the surface of which is mainly occupied by Sl. (*Salicornia herbacea*). The centre of the grid bears a uniform carpet of Sl. G. (*Salicornia herbacea*, bright crimson in colour, mixed with *Glyceria maritima*). A narrow strip on the left is occupied by G. Sd. (*Glyceria* with *Suæda maritima*), while the upper portion of the grid is part of an area of Sd. G with Sl. scattered through it.

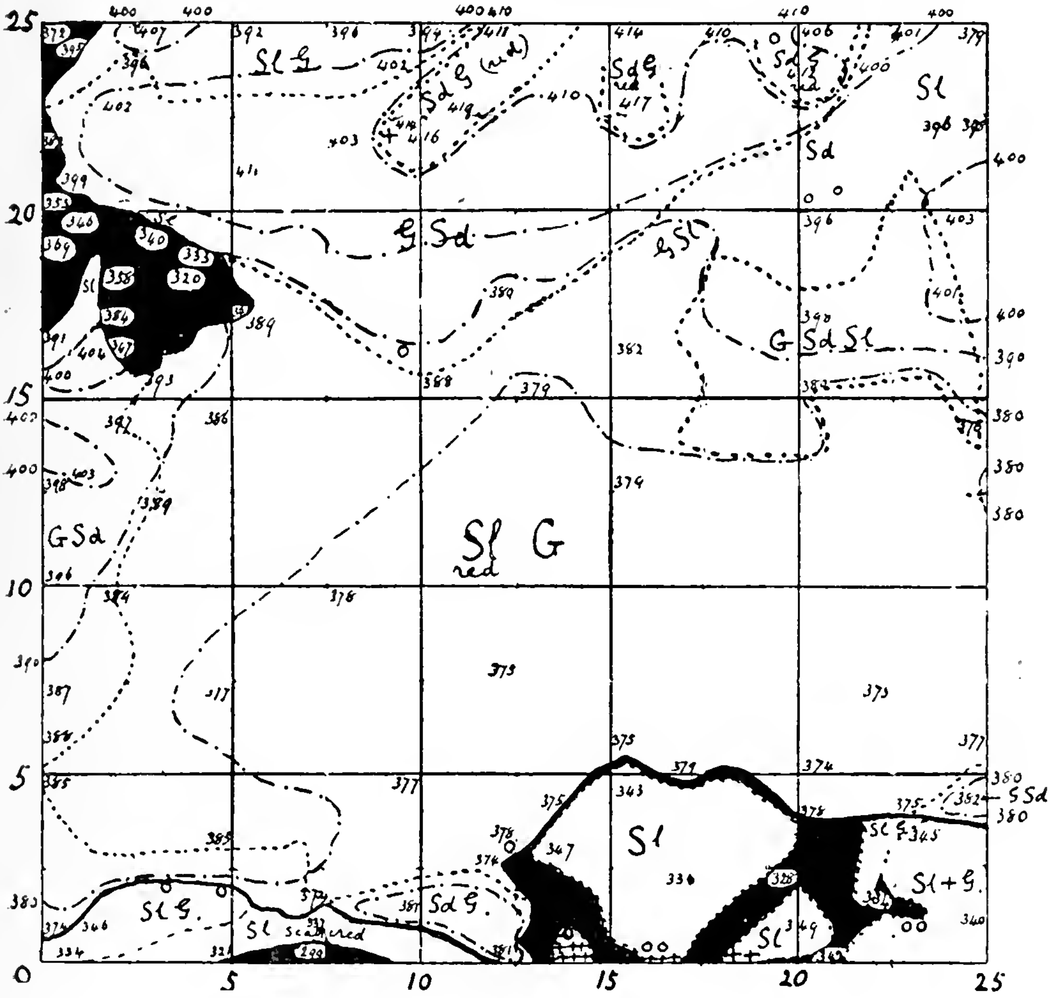


Fig. 80. A mapped Gridiron with levels and contour lines.
Two sets of " bars " at right angles to one another are represented.

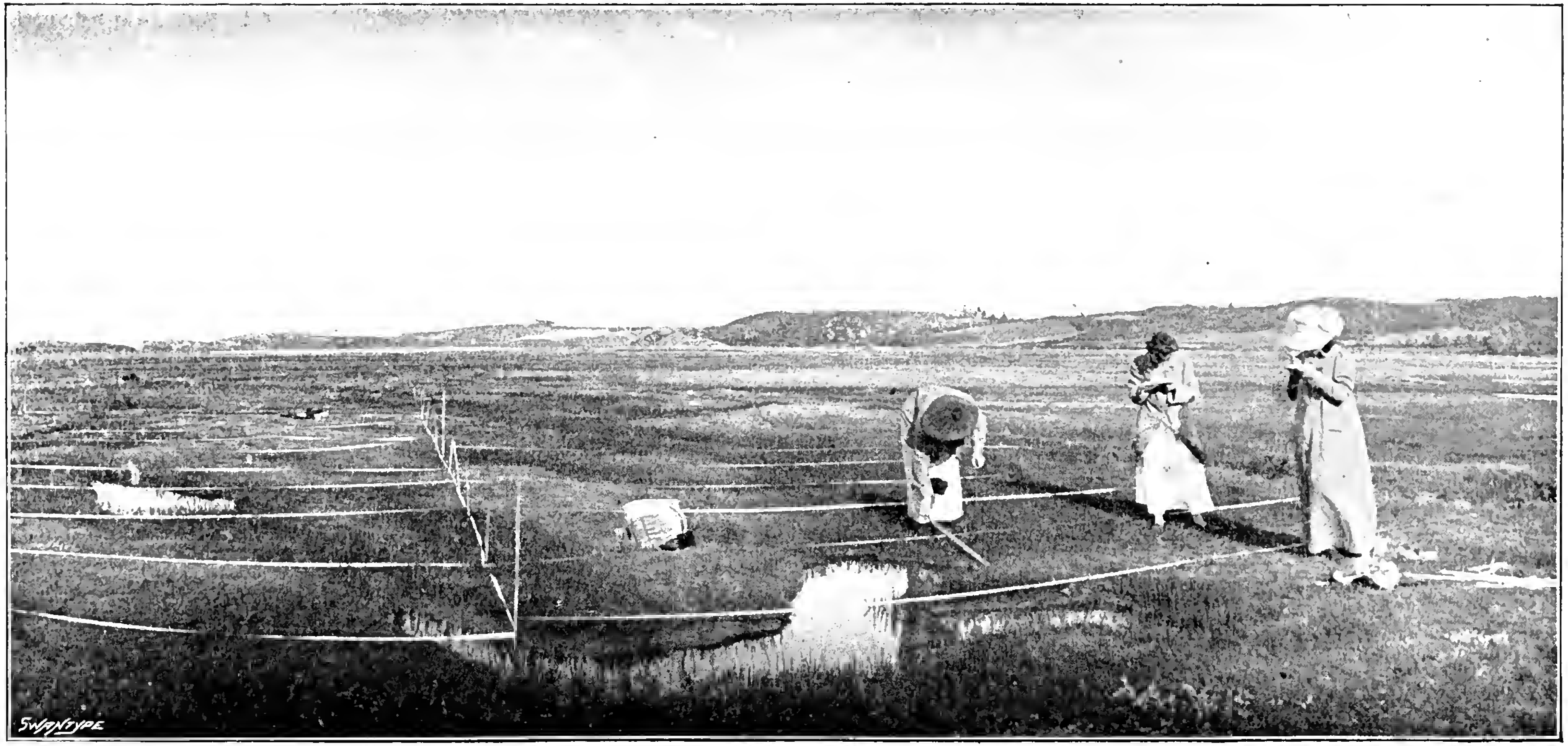
Fig. 80 shews that the surface of the channel is partly bare and consists partly of slipped masses of mud covered with Sl. or Sl. G. at a considerably lowel level than the general Sl. G. plain, but considerably higher than the surface of bare mud, and with a few scattered plants of *Obione portulacoides* (0) and *Salicornia radicans* (+) on their edges. It also shews that the Sl. G. plain is practically flat for most of its extent, and slopes gradually up towards the left and towards the top to higher ground occupied by G. Sd. In the top right hand corner are patches of intermediate associations in which G., Sl., and Sd. are variously mixed. At the extreme top of the square three spurs, bounded by the 410 contour, project from the higher ground which is out of the map. These spurs are covered with Sd. G. in which the Sd. has a characteristic red (madder) colour. A few plants of *Obione* and *S. radicans* are found on the edge of the higher ground, while the Sl. G. plain is quite destitute of them. Part of an irregular pan, largely bounded by Sl. and Sl. G., and whose bottom is at a distinctly higher level than that of the channel at the foot of the grid, appears in the top left-hand corner.

It is not part of the purpose of this paper to discuss further the features exhibited by Fig. 80. But enough has been said to shew the kind of features that can be shewn, and the way in which in the detail of the information given on the smaller scale map can be filled in.

Plate XI. is from a photograph shewing the characteristic vegetation of the Bouche d' Erquy. It is taken from near the extreme corner of the square and grid represented in Figs. 78 and 80, looking over the square with the grid in the foreground. Most of the features shewn in the figures can be identified in the photograph. The G. Sd. association is on the higher ground and appears lighter, while the G. Sl. is on the lower grouud and appears darker.

A single grid-map can be used as a detailed sample of vegetation shewn on a larger map, or a connected system of grids can be constructed, as in the case of the system of squares previously described, where it is desirable to make a detailed survey of a larger area. Fig. 80 belongs to such a system, which was chosen to exhibit the nature of the ground and the vegetation connected with a series of "pans" and ridges extending from the red Sl. G. plain (of which the part shewn in the figure is one extremity) to certain deep supply channels, some 130 feet away.

Finally it may be again remarked that the gridiron method is only available on fairly flat or gently undulating ground, and that it is probably not worth applying except in such cases as that illustrated, where there is a definite and considerable variation of the vegetation in the space of a few feet, in correlation with a similar variation of some one or more physical features. In the case



OLIVER AND TANSLEY.—METHODS OF SURVEYING VEGETATION.

Photograph of part of the Bouche d'Erquy. The gridiron under survey is the one represented in Fig. 80. The rest of the square shewn in Fig. 78 lies behind, away from the observer. The system of grids to the left is not shewn in the Figures.

illustrated, the level of the soil surface, the bottom-water-level, and probably the salt content, are the correlated physical features which determine the distribution of the vegetation. Similar conditions often obtain in fresh-water marshes where the bottom water level is close to the surface.

CHROMATIC ADAPTATION.

FACTS AND THEORIES CONCERNING THE ADAPTATIONS OF PLANTS TO DIFFERENCES OF ILLUMINATION.

By F. F. Blackman.

A CERTAIN human interest attaches to the different adaptations by which plants attempt to rise superior to unfavourable variations in their environment.

The green land-plant, as a generalised structural type, is an expression of the necessity, under which it always is, of obtaining energy by the absorption of radiation from above. Nearly all the striking variations of this type which the plant-world displays are correlated, however, not with variations in illumination, but with special variations in the water supply of their particular habitat.

The absence of striking adaptations to different intensities of natural illumination ceases to surprise, when one realises the fact that no plant under existing natural conditions can utilise more than a small fraction of the energy of the direct sunshine which may fall upon it. There may be enough chlorophyll in a leaf to absorb 20% to 25% of the sun's radiation[1] in addition to the amount absorbed by the sap, cell-walls and protoplasm, but the actual CO_2-assimilation performed cannot, under natural conditions, represent more than about $\frac{1}{6}$th of the available energy. The reason for this is that, in nature, assimilation is limited not by light but by the available CO_2-supply, because sufficient CO_2 cannot diffuse into a leaf from an atmosphere containing only three parts of CO_2 in 10,000. The writer has shown that an active leaf exposed both to the bright diffuse light and to the direct sunshine of a brilliant August day can absorb enough energy *in a form available for CO_2-assimilation* to produce a photosynthesis of six litres of CO_2 per square metre of area per hour. Now, from determinations made

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[1] Timiriazeff. The Cosmical Function of the Green Plant. Proc. Roy. Soc., Vol. 72, p. 449.

by Horace Brown[1] of the gain in weight[2] of actively assimilating leaves, it would seem that a leaf in the open air is limited to acquiring about 800cc. of CO_2 per square metre of area per hour.

It is clear then that the energy available for assimilation in sunshine is so much in excess of the supply of raw material available for the leaf to work up, that comparatively feeble light is adequate for all assimilatory possibilities.

We should not expect, then, to find adaptations for making the most of the light that reaches a leaf, except when the natural illumination falls below a certain low level. Confirmed "shade-plants" show a certain amount of structural adaptation in this direction, but the differences between sun-leaves and shade-leaves are also partly correlated with the function of transpiration. The most efficient illumination-adaptations seem to take the form of lens-like arrangements of cell-walls which focus upon the chromatophores such light as is available. It is very striking that, in no case, does a land-plant heighten its assimilating power by developing in its chromatophores a pigment which will absorb some of the rays of light which chlorophyll transmits unutilised.

With plants growing submerged in various depths of water the state of things is quite different. Water is a blue liquid, and the deeper the water the more the rays of the red end of the spectrum are absorbed. Spectroscopic observation has shown that at fourteen metres below the surface of the sea the light that penetrates is mainly composed of green and blue, feeble in yellow, and lacks red entirely.[3] These red rays are just those most efficient in CO_2-assimilation and this loss, combined with loss of light of all kinds by reflection from the troubled surface of the sea, and by absorption by fine suspended particles brings the efficiency of the light so low that it falls below the intensity corresponding to the quantity of CO_2 available for assimilation.

An adaptation is then profitable to promote the assimilating activity of sea-weeds at these depths, and we find that this takes the form of a brilliant red colouration, which provides an efficient absorber of the green and blue rays that preponderate in these depths. Between the red deep-water algæ and the superficial algæ which

Horace Brown. Presidential Address, Chemical Section of British Association, 1899.

Sachs deduced from one of his experiments that 1400 cc. might be absorbed, but there are good reasons for regarding this number as too high.

Engelmann. Couleur et Assimilation. Arch. Néerlandaises. Tome xviiii. 1883.

are green, and depend on the same rays as plants in air, there is found an intermediate zone of brown algæ which make up for some deficiency of red light by an added power to absorb, to a certain extent, green and blue rays.

These three coloured algal strata, then, tend to possess complementary colours to the lights in which they are bathed, and thus to arrive at the maximum absorptive efficiency for their respective habitats.

There are yet other types of algæ which are not pure green in colour, the Cyanophyceae or blue-green Algæ. These are all minute forms and show a great range of colouration, being purple, violet, brown, yellow, olive green or blue in different cases, though the most usual colour is blue-green. This group possesses at the present day a much greater mobility of colouration than the other groups.

It is, of course, well known that the red, brown and blue-green algæ all contain chlorophyll in their chromatophores, but that these organs contain, in addition, red, brown or blue pigment which entirely or largely masks the green colour. These adaptational pigments all differ profoundly from chlorophyll in chemical constitution (being usually held to be of a proteid nature) and all are soluble in water and diffuse readily out of the dead cell. These secondary pigments can be readily discharged by dipping a red, brown or blue-green alga in boiling water, after which the chlorophyll green stands revealed.

We now come to the interesting point that as these adaptational pigments are *additional* and not substitutional, it would appear that a red alga snould be a more efficient absorber of light than a green one, not only at great depths, but also at the surface. Nevertheless the red algæ have not replaced the green forms at the surface, but are only found there in small numbers. The probable explanation is that assimilation at the surface of the sea is limited by the amount of CO_2 attainable, as it is on land, and that therefore additional power of absorbing light-energy is of no assimilatory advantage. The red algæ have no advantage over the green at the surface, though at a sufficient depth the green alga will be completely out-classed. Here then in the case of marine algæ we have what Engelmann has called "complementary chromatic adaptation" arrived at in the course of evolution.

An extremely interesting case has recently been carefully investigated by Gaidukov[1] in which "complementary chromatic adaptation" can be brought about in the course of a few weeks, and all the details of the change have been followed by him.

He found that filaments of *Oscillaria* possess the power of changing their colour when grown behind coloured glass or coloured solutions and always in the direction of taking on the complementary colour to the light which they are made to exist in, and so absorbing it more efficiently. According to the views here put forward, this would be a distinct biological gain only if the light were so weakened that it became the limiting factor in assimilation. In this connection it is significant to note that Gaidukov's cultures were grown inside the laboratory, though within a yard of a big south window, and the colour screens used were mostly deeply coloured.

Oscillaria sancta, which is violet in white diffuse daylight, was first investigated, and pure cultures growing on earth or agar in Petri dishes, were kept in the different coloured lights. After one to four weeks they were found to have taken on the complementary colour, being greenish in red light, blue-green in yellow brown light, and yellow-brown in blue light. During this time the cultures had grown rapidly, so that the final colour was due chiefly to newly-formed cells. Another species, *O. caldariorum*, which is blue-green in daylight, gave quite similar colour-changes.

When a filament is transferred from one light to another which is spectrally remote from it, as from red to blue, it does not at once begin to assume its correct final reddish colour, but passes in succession through a long series of intermediate stages of colouration, being the colours approximately complementary to the intermediate spectral lights in order. This series of colours is given by Gaidukov as (1) sky-blue, (2) blue-green, (3) verdigris-green, (4) grey-green, (5) whitish-grey, (6) violet to brown-violet, (7) brown (8) orange to reddish. *O. sancta* in ordinary white light happens to have colouration 6. Transferred to red light it passes *up* the series to 1. Placed in blue light it passes through 7 to colour 8. *O. caldariorum* happens in white light to have colour 2 and transferred to green light it passes *down* the series to 7 or 8.

As the individual cells are multiplying all the time it may be that each generation only takes a short step in the direction of the

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[1] Gaidukov. Über den Einfluss farbigen Lichts auf die Farbung lebender Oscillarien.
(*a*). Anhang zu Abh. k. Preuss. Akad. Wiss. Berlin, 1902, v.
(*b*). Ber. deut. bot. Gesell., Bd. xxi., Oct., 1903.
(*c*). ,, ,, ,, Nov., 1903.

final colour, and so the culture goes on gradually improving its biological position.

It is interesting to note that if the cultures were brought back to white diffuse light they retained for months the colour that they had acquired behind the coloured screen, and even the new cells formed thus under natural illumination exhibited the acquired colour. The ultimate fate of these nascent new "forms" has not yet been announced.

As has been pointed out, white daylight probably supplies sufficient of all the spectral radiations to allow *any* of the *Oscillaria*-colours to assimilate abundantly, so that there would be no *assimilatory* gain in the new forms reverting to their ancestral colour.

Gaidukov's first paper givés very excellent full details of the colour-changes and is illustrated with coloured figures of the filaments themselves, of the colour-screens and of the absorption spectra of the different coloured pigments. Also there are given detailed measurements of the change of absorption for different rays as one pigment passes into another. The author draws attention to the fact that each stage of colour-progression passes by imperceptible gradations into the next and that here, as elsewhere, *Natura non facit saltum.*

The different shades of colour are due to modifications, not of the chlorophyll in the chromatophore, but of the water-soluble proteid pigment which is here called the adaptational pigment. If abundant this completely masks the green colour.

It has generally been held that the blue water-soluble pigment of the Cyanophyceae belongs to the same class as the red and brown pigments of the Rhodophyceae and Phaeophyceae but it would appear now that when the blue-green *Oscillaria* becomes red under the influence of green light it developes a pigment identical with that of the Rhodophyceae.

The characteristic secondary proteid pigments of these three groups of algæ can be crystallised from their watery solutions so that in time we may hope to know their chemical relation. The watery solutions are slowly decomposed by light but show no indication of complementary colour change outside the living cell.

It is interesting to note that the pure green algæ and the higher plants which have evolved from them, appear to have no water-soluble proteid pigment in their chromatophore though to the best of the writer's knowledge such a pigment has never been carefully looked for. If so, they would seem to be devoid of, or to have lost, the material basis for carrying out complementary chromatic adaptation, for chlorophyll itself is apparently essentially the same substance throughout the whole range of CO_2-assimilating plants.

This experimental production of complementary chromatic adaptation takes rank as a quite new phenomenon, both from a physical and from a biological point of view. The production of the converse phenomenon, "*sympathetic* chromatic adaptation" is however known physically in a few cases and has often been observed biologically in such cases as the "protective colouration" of insects.

In the latter we find the insect developing a pigment of the same colour as the light which is shining on it from its enviroment, while of the former we have a very good example when a surface of mixed chlorides of silver and other metals, illuminated by a spectrum, gives an image of the spectrum in approximately the true colours in all its parts. This phenomenon, extraordinary as it appears at first sight, has been satisfactorily explained by Wiener.[1] It is due to the salts being sensitive to all wave-lengths of light and giving with white light an appearance of darkening due to a mixture of all possible coloured particles. When now the red of the spectrum shines on this it slowly decomposes all the particles that absorb the red rays. The only particles of the mixture that will not absorb red light are of course the red particles and these alone remain and give a red patch of pigment. So in the blue part of the spectrum all the particles except the blue are decomposed and a blue patch results: similarly with intermediate parts of the spectrum.

The colouration changes studied by Keeble and Gamble[2] in *Hippolyte varians* seem to be quite different and much more complex.

These reactions of chlorides may throw light on sympathetic chromatic adaptation but it is not clear how they help the case of complementary chromatic adaptation. This latter is not a *direct* complementary reaction of the coloured cell to the new coloured light as is indicated by the long series of colour stages that must be gone through before the final complementary colour is arrived at.

It is to be hoped that investigation may soon bring us a fuller comprehension of this new and important chromatic adaptation.

[1] Wiener. Farbenphotographie, Annalen d. Physik. u. Chemie, Bd. 55, 1895.

[2] Keeble & Gamble. Quart. Journ. Mic. Sci., Vol. 43; also Phil. Trans., Royal Society, Vol. 196, B.

NOTE ON FLORAL ANOMALIES IN SPECIES OF CERASTIUM.

[With Figs. 81-84.]

1.—Secondary Whorl of Carpels in *Cerastium quaternellum* Fenzl.

DURING some recent work on different species of the group of Alsineæ in Caryophyllaceæ, I have encountered some anomalies which seemed sufficiently interesting to justify special notice. In *Cerastium quaternellum* Fenzl (Moenchia erecta Sm.), a very ephemeral little species which is common in spring on dry sandy banks throughout the country, the above departure from the normal type was twice observed.

The flowers in this species are tetramerous, sepals in two whorls, petals, stamens and carpels being in regular alternation. The ovary consists of four loculi, with two rows of campylotropous ovules in each. The placental column reaches to about two-thirds the height of the ovary and on it the ovules are inserted in eight rows, about seven in each, in basipetal succession. The loculi are of the usual Alsinean type. The dissepiments fuse with the placental column, making the ovary septate for the length of the same. Above the column, owing to the widening of the ovary, they separate out, to fuse again as it narrows in the basal region of the styles. The tissue of these dissepiments soon breaks down, becoming loose and spongy and more or less lacunar. On the placental side it widens out, forming a sort of cushion, functioning as conducting tissue for the pollen tubes, and persists as the so-called "Leisten" mentioned by Eichler.

The connection with the carpellary wall is gradually reduced to a few strands of parenchyma, which completely break down after fertilization, leaving a free central placenta. The styles shrivel up after the passage of the pollen tubes. The anomalous structure in the ovary in question is shown in longitudinal sections, slightly oblique Fig. 81 (s.w.c.) which is a camera lucida drawing of the most median section of a complete series through the entire ovary.

The secondary whorl of carpels (Fig. 81, s.w.c.) is seen occupying the extreme apex of the placental column, its vascular strands being in continuity with those which supply the primary whorl. By a careful comparison of the whole series, the anomaly is seen to consist of three carpels with three styles (Fig. 81, St.), forming three loculi with one campylotropous ovule in each (Fig. 82). The placental column is well seen (Fig. 1, p.c.), the ovules being inserted at its apex, which is what one would expect. The dissepiments are of the usual spongy character, thickening in juxtaposition to the placental column and forming partial septation only in connection with it. They are also connected by a few strands of tissue with the carpellary walls. The epidermis of the secondary carpels is lignified at the apex (Fig 81, lig. e), as is the case with those of the primary whorl.

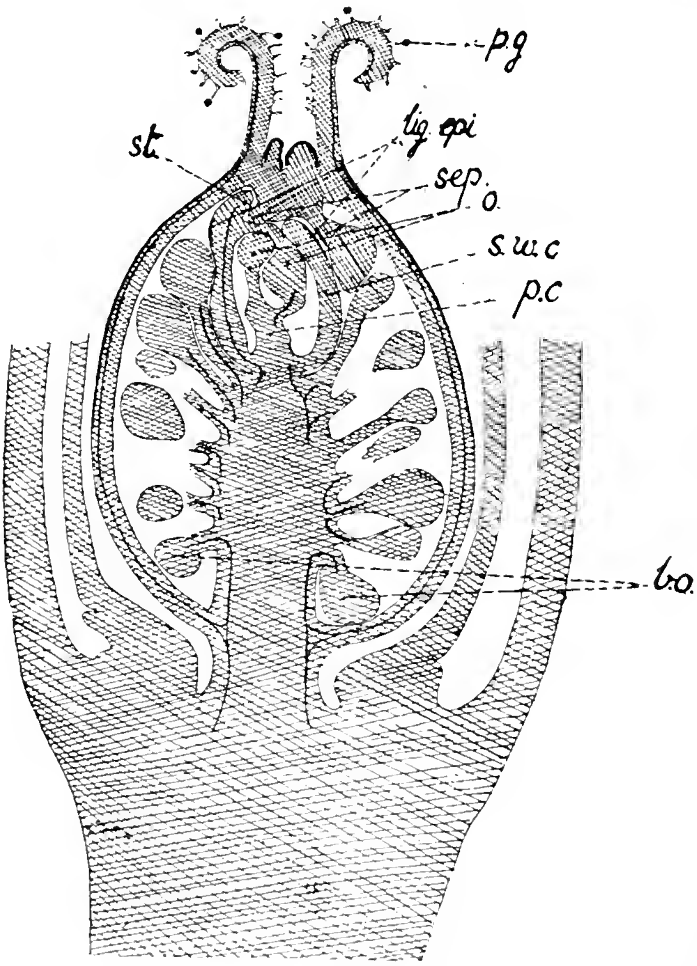

Fig. 81.

Cerastium quaternellum. Slightly oblique longitudinal section of ovary of anomalous flower.

So that, apart from the great reduction in the number of the ovules, which is clearly in relation to space and food-supply, this secondary ovary is seen to be an exact replica of the primary one. In the stage seen, the styles cannot extrude, but that would not preclude fertilization, as any unannexed pollen-tubes could find their way down the conducting tissue of the primary ovary to the styles of the secondary one.

The ovules, of course, are much younger, apparently in the embryo-sac mother-cell stage. They seem quite normally developed, though their position is morphologically affected by their straitened circumstances. This abnormal pressure has also caused the depressed position of the lowest ovules in the primary carpels (Fig. 81, b.o.) These are normally erect, with the funicles growing at a similar angle to those in the same vertical row.

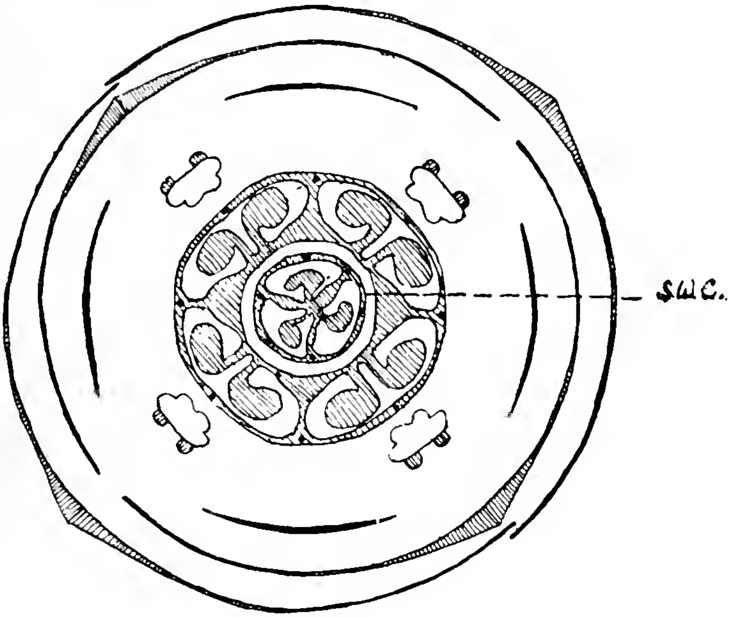

Fig. 82.

Cerastium quaternellum. Floral diagram of anomalous flower.

The only analogy I can find with this peculiar development is the case of *Punica granatum*[1] where one or sometimes two subsequent oligomerous whorls of carpels in the inferior ovary, are of normal occurrence.

Payer[2] considered the placental column in the Caryophyllaceæ to be of an axillary nature. The above anomaly, if such evidence can be accepted, seems to lend some support to this view.

2.—Two Nucelli in One Ovule.

Cerastium glomeratum. Thuillier.

This ovule was a very conspicuous feature in a section through an ovary of the plant in question, on account of its abnormal size. The first idea was of two ovules grown together, but investigation proved that to be out of the question.

Throughout the whole series of sections only one outer and one inner integument are seen to enclose both nucelli. The divi-

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[1] Eichler. Blütendiagramme, p. 489.

[2] Payer. Organographie, p. 326, Tab. 71, 73 and p. 344, Tab. 70.

sion between these latter is clearly shewn, with no trace of extraneous tissue. The position of the micropyle is normal, but the inner integument shows an abnormal stretching (Fig. 83, i.i.) to adapt itself to the increased surface; the funicle also is distinctly single throughout. The ovule is cut tangentially (Fig. 84). The two nucelli are quite distinct (Fig. 83, n.) enclosed in an inner integument (Fig. 83, i.i.) each containing a fully developed embryo-sac (Fig. 83, e.s.) in the pre-fertilization stage. The two synergidæ (Fig. 83, syn.), oosphere (Fig. 3, Oos.), and definitive nucleus (Fig. 83, d.n.) are seen in the series of sections.

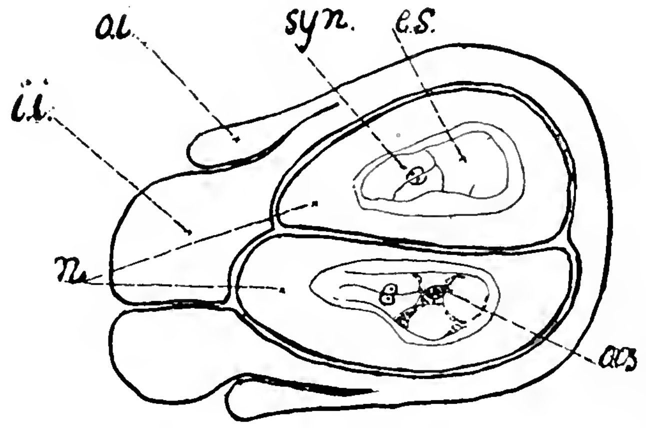

Fig. 83.

Cerastium glomeratum. Tangential section of anomalous ovule.

In *Cerastium quaternellum* an exactly similar case was seen, the division of the nucelli being as plainly marked, with both surrounded by one inner integument.

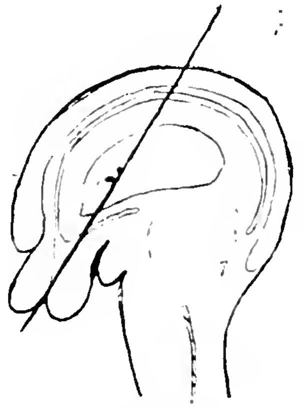

Fig. 84.

Cerastium glomeratum. Median section of anomalous ovule shewn in Fig. 83. The line shews the plane of section of Fig. 83.

Hofmeister[1] describes the occurrence of two nucelli in an

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[1] Hofmeister, W. Neuere Beobachtungen über Embryobildung der Phanerogamen. Jahrb. f. wiss. Bot., Bd. I., pp. 82-188, 1858.

inner integument as of frequent occurrence in *Morus alba.* Braun[1] ascribes this to a forking of the end of the ovular rudiment (Eisprösschen). Miss Benson and Miss Sanday[2] have found one such case in *Carpinus Betulus.*

Royal College of Science. L. S. GIBBS.

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[1] Braun, A. Ueber Polyembryonie und Keimung von *Cælobogyne*, ein Nachtrag zur Abhandlung über Parthenogenesis bei Pflanzen. Abhandl. König. Akad. d. wiss., Berlin, pp. 109-263 1859.

[2] Miss Benson's and Miss Sanday's slide.

RESEARCHES ON THE VASCULAR STRUCTURE OF CYCADS.[1]

THE botanical investigator of self-complacent attitude who imagines that because he has worked at a subject for many years from (to him) every conceivable point of view, with the apparent result of having exhausted the subject, forgets that his point of view can be but a partial and limited one and that often in the case of the most hackneyed material, another type of mind regarding the subject in turn from *his* standpoint, may unearth treasures from the same old field which the previous investigator, for the reason above mentioned, failed to find.

This principle is well illustrated by the recent appearance of Dr. Matte's thesis bearing the above title, a thesis whose chief recommendation would seem to be that it presents a *tout ensemble* of admirably marshalled facts most of which were already familiar as such, but had never been seen, at least from this aspect, in such a clear light, nor in such well-ordered array. The author exhibits the power, rare in our day, of extracting from the chaos of scattered facts of detail one overlying generalisation,— of creating unity out of diversity, a method of procedure which shou'd be the chief aim of all scientific investigation.

These well-ordered facts, then, deal with the course and structure of the vascular bundles of Cycads, the greatest emphasis being laid on these phenomena as they occur in the "meriphyte" or foliar organs of the plant, the vascular structure of the axis being (in our opinion, rightly) regarded as of secondary origin and importance.

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[1] H. Matte. "Recherches sur l'Appareil Libéro-ligneux des Cycadacées," Caen, 1904.

The first section of the paper treats of the course of the bundles from the pinnae into the rachis of the leaf and downwards into the petiole and leaf-base. In order that this course may be accurately traced, great insistance is made on following, not so much the entire bundle, as the individual protoxylem-groups. What we most admire is the ingenuity displayed in demonstrating how all the varied modes of arrangement of the bundles of the petiole, from the simple system of *Cycas or Dioon* to the highly complex grouping in *Encephalartos*, are merely modifications of the characteristic Ω. The clarity and profuseness of diagrammatic representation of this feature are indeed charming. It seems to us that the writer sheds here, in his own way, a light which never was on any other handling of this particular subject. It is always exhilarating to observe simplicity reappearing out of dire complexity. From the resemblance of the petiolar arc of bundles in *Bowenia* to that in *Angiopteris evecta*, and in view of the schizogenous development of the mucilage-canals in both this fern and Cycads, he suggests the possibility of an actual relationship between the latter group and Marattiaceæ.

The second section deals with the "Flower." The morphology of the sporophyll of the cone and its parts is discussed, and the course and structure of the bundles as they are found both in the sterile lamina, in the basal stalk, and in the sporangium (ovule) are most carefully and interestingly described. The course and character of the bundles in the axis of the cone also receives special attention. With Solms-Laubach, Scott and ourselves the author agrees in regarding the vascular structure of the cone as more primitive and ancestral in organisation than that of the vegetative part of the plant. What we more particularly rejoice to see is the author's recognition of a probably homological correspondence between the inverted or concentric strands, situated just outside the central cylinder of the peduncle in *Encephalartos* and others, not only with the "extrafascicular" strands in the vegetative stem of *Cycas*, *Encephalartos*, &c., but also with the outer ring or rings of the Medulloseæ.

Considerable attention is devoted to the vascular supply of the seed; the author describes two distinct systems: the integumental bundles, corresponding (in accordance with his acceptance of the ovule as the homologue of a leaflet) to the nervation of a leaf-segment, and the "perinucellary" strands representing a recent addition to the vascular system and which have been "accidentally inserted" on the latter; the "perinucellary" bundles are admitted to be integumental and are compared with the inner integumental system in the seed *Lagenostoma*. Reference is also made to Miss Stopes' work in this directon on Cycads. In order to account for the fact of the bundles in the cupule or outer envelope in Cycads being orientated with inwardly-directed xylem, involving, as the author concludes, the situation of the sporangium on the *upper* surface of this envelope, he suggests that, starting out from the primitive Filicineæ in which the sporangia were probably situated at the termination of the veins, *i.e.* on the margin of the leaf, two new types have become differentiated, *viz.* the one in which the sporangia became, as in modern Ferns and Coniferæ, displaced on to the lower, and the other, in which they were relegated to the upper surface, as in Cycads.

In the pith of the peduncle of *Encephalartos villosus* the author discovered an intrafascicular reduced ring of bundles such as we ourselves found and described in the cone-axis of *Ceratozamia latifolia*; he agrees with us in believing that this ring of strands corresponds to that occurring in a similar position in the stem of *Medullosa Solmsii*, *M. porosa*, and *M. stellata*.

Section 3 is occupied with the study of *seedlings* and their anatomy. From certain rather abstruse facts connected with the mode of union of the cotyledonary strands with the poles of the xylem-plate of the root in *Dioon* and *Cycas*, as well as from one or two other phenomena, the author agrees with several writers in regarding the root as an independent new organ, inserted on the base of, and growing in the same straight line with, the hypocotyl, and not as a mere prolongation of the latter.

As regards the so-called "abnormal" strands possessing *inverted*, as well as normal, orientation of their parts, thus constituting *concentric* steles situated outside the central cylinder, which Gregg (true type of the old-fashioned and, we hope, departing anatomist) described, without ever attempting to explain, in the root of *Cycas Seemanni*; which we ourselves observed in the hypocotyl of several Cycads; and which our author mentions as occurring in *Cycas siamensis;* these strands, we say, are admirably treated of and receive at last that due recognition of their merits as part of the *normal* structure, inherited, out of the dim past, from a tissue which was probably none other than the second "Schlangenring" or the zone of concentric bundles or steles in the stem of such a plant as *Medullosa Solmsii* or *M. stellata*.

The observation, however, of the author which, in our opinion, crowns all in adding weight to the already strong evidence in favour of the Medullosean descent of the Cycadaceæ, is that of the occurrence in the hypocotyl of *Encephalartos Barteri* (seen, we believe, for the first time in the history of the study of modern Cycads) of a *central cylinder consisting*, not of the usual collateral vascular structure, *but of a group of three or four concentric strands or steles*, quite comparable to the group of steles which constitute the vascular tissue of the stem of *Medullosa anglica* as described by Scott. We have *always* maintained and believed, in contradistinction to the opinion of Scott (who has ever held to the *monostelic* derivation of the Cycadean central cylinder), that in *all* Cycads the central vascular ring of collateral strands has been derived by descent from a cylinder of *concentrically-constructed steles*, such as obtains in the Medulloseæ, by means of the extinction of the inner inverted portion of such steles. This view is completely endorsed and supported by Dr. Matte. It should be remembered that it is precisely in the seedling plant, and especially in its hypocotyledonary region that we might expect the appearance of ancestral traits. If the structure occurring in the hypocotyl of *Encephalartos Barteri* represent indeed, as we firmly believe, the normal adult structure of the stem in the ancestors of this genus; then it follows that precisely the same must be true in the case of *all* other Cycads, whether mono- or pleio cylindric.

In conclusion, we should like to congratulate the author on an original and illuminating contribution to our knowledge of the structure and affinities of this remarkable group of plants.

Kew, W. C. WORSDELL.
November 1st, 1904.

THE LONDON BOTANICAL SOCIETY.

THE forty-ninth Meeting of this Society was held in the Biological Lecture Room of the Royal College of Science at 4.30. p.m., on Monday the 28th ult., Dr. D. H. Scott, F.R.S. in the Chair.

Dr. F. E. Fritsch read a paper on the structure of the investment in Cyanophyceae. It was shown that within the external mucilaginous envelope of an *Anabaena* two membranes could be distinguished around each cell, as soon as the cells began to pass over to the sporogenous condition. Of these the inner investment extends completely around the protoplast, whilst the outer one (the cell-sheath) envelopes the cell as a cylindrical sheath open at each end; the transverse septa are thus formed by the inner investment only, whilst laterally both envelopes of the cell can be distinguished. When a cell divides, the cell-sheath is split into two fresh sheaths by the development of a new intercellular septum; each cell thus has an individual cell-sheath. As the spores mature they become widely separated from one another, whilst the cell-sheath closes in round the open ends, forming a complete exospore, the endospore being constituted by the inner investment. The cells of *Gloeocapsa* exhibit the same two envelopes, which here completely envelope the cell; the cell-sheath is split during division, but the daughter-cells become widely separated by the excretion of intercellular mucilage, and the cell-sheath closes round and forms a complete investment (cf. mature spores of *Anabaena*). In *Oscillaria* the cell-sheath is no longer split during division, but forms a coherent whole round the filament; *Lyngbya* only differs from *Oscillaria* in the presence of a consistent external mucilage-sheath. In the higher heterocystous forms (e.g. *Tolypothrix* and *Rivularia*) however the actual filament (within the external sheath) is provided with a cell-sheath, which is only in part coherent and shows a very marked moniliform structure. In young stages the inner investment is alone recognisable; it is regarded as a modified plasmic membrane of a viscous, gelatinous nature, and it is possible that the intercellular protoplasmic connections of many observers are merely due to contractions, produced in the gelatinous transverse portion of the inner investment during staining.

Professor Fujii of Tokio, Japan, exhibited preparations of the spermatozoids of *Ginkgo biloba* and gave some account of their normal and abnomal structure and of their behaviour in fertilization. He also described the germination of the pollen-grain in this Gymnosperm and criticised the retention of the terms "stalk" and "body" cell as wholly inappropriate.

Professor F. W. Oliver exhibited a new seed from the Lower Coal-Measures recently received from Mr. J. Lomax. He stated that it was the smallest seed that, had come under his observation from this horizon, and whilst probably having affinity with the Lagenostoma-group it shewed certain interesting points of agreement with *Gnetopsis elliptica* as described by the late Monsieur B. Renault.

HONOURS AND POST-GRADUATE COURSES IN BOTANY AT THE UNIVERSITY OF LONDON.

CONSIDERABLE progress has been made with the three-year programme of lectures drawn up last year, since the publication in this Journal (Vol. II., p. 212, November, 1903) of a note on the scheme.

After the conclusion this time last year of Mr. A. D. Hall's admirable course on "The Relation of the Plant to the Soil in which it grows," Dr. Scott's course in the second term at University College, on "The Lycopsida," attracted large audiences of fifty or sixty. In the summer term Professor Green's course at Chelsea on "The Metabolic Processes of Plants" was also well attended.

During the present term the Director of Kew Gardens has given a course of four lectures at Chelsea on "The Problems of Modern Botany," dealing mainly with questions connected with the activity of chlorophyll. In the latter half of the term, Mr. W. G. Freeman, of the Imperial Institute has delivered an attractive course of six lectures on "Current Investigations in Economic Botany" also at Chelsea.

Next term Mr. V. H. Blackman will lecture at University College on "The Origin of the Ascomycetes" beginning on Wednesday, January 18th, at 5 p.m., while in the summer term courses will be delivered by Dr. F. F. Blackman, of Cambridge, on "The CO_2-economy of Plants" and by Dr. A. B. Rendle, of the British Museum, on "Tubiflorae." The lectures are all free.

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